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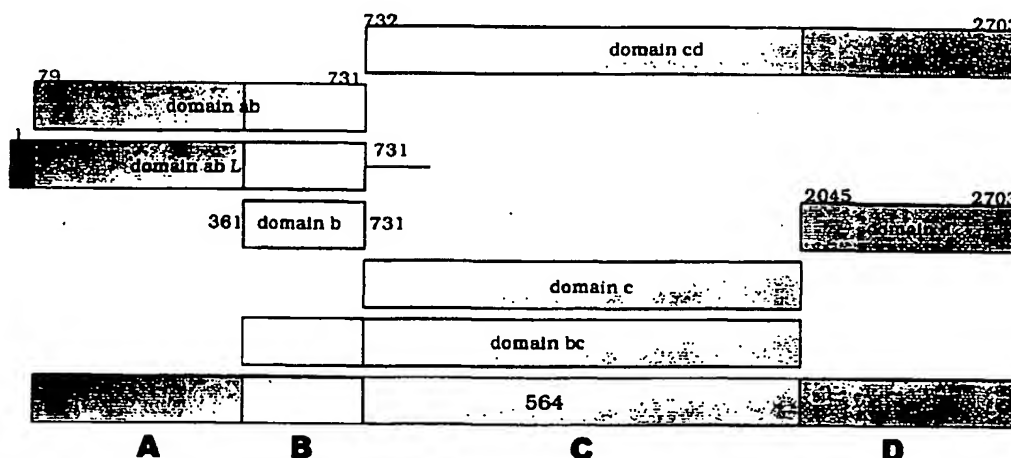
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(54) Title: HETEROLOGOUS EXPRESSION OF NEISSERIAL PROTEINS



(57) Abstract: Alternative and improved approaches to the heterologous expression of the proteins of *Neisseria meningitidis* and *Neisseria gonorrhoeae*. These approaches typically affect the level of expression, the ease of purification, the cellular localisation, and/or the immunological properties of the expressed protein.

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## HETEROLOGOUS EXPRESSION OF NEISSERIAL PROTEINS

All documents cited herein are incorporated by reference in their entirety.

### TECHNICAL FIELD

This invention is in the field of protein expression. In particular, it relates to the heterologous  
5 expression of proteins from *Neisseria* (e.g. *N.gonorrhoeae* or, preferably, *N.meningitidis*).

### BACKGROUND ART

International patent applications WO99/24578, WO99/36544, WO99/57280 and  
WO00/22430 disclose proteins from *Neisseria meningitidis* and *Neisseria gonorrhoeae*.  
These proteins are typically described as being expressed in *E.coli* (i.e. heterologous  
10 expression) as either N-terminal GST-fusions or C-terminal His-tag fusions, although other  
expression systems, including expression in native *Neisseria*, are also disclosed.

It is an object of the present invention to provide alternative and improved approaches for  
the heterologous expression of these proteins. These approaches will typically affect the  
level of expression, the ease of purification, the cellular localisation of expression, and/or the  
15 immunological properties of the expressed protein.

### DISCLOSURE OF THE INVENTION

#### *Nomenclature herein*

The 2166 protein sequences disclosed in WO99/24578, WO99/36544 and WO99/57280 are  
referred to herein by the following SEQ# numbers:

Application	Protein sequences	SEQ# herein
WO99/24578	Even SEQ IDs 2-892	SEQ#s 1-446
WO99/36544	Even SEQ IDs 2-90	SEQ#s 447-491
WO99/57280	Even SEQ IDs 2-3020	SEQ#s 492-2001
	Even SEQ IDs 3040-3114	SEQ#s 2002-2039
	SEQ IDs 3115-3241	SEQ#s 2040-2166

20 In addition to this SEQ# numbering, the naming conventions used in WO99/24578,  
WO99/36544 and WO99/57280 are also used (e.g. 'ORF4', 'ORF40', 'ORF40-1' etc. as  
used in WO99/24578 and WO99/36544; 'm919', 'g919' and 'a919' etc. as used in  
WO99/57280).

The 2160 proteins NMB0001 to NMB2160 from Tettelin *et al.* [*Science* (2000) 287:1809-1815] are referred to herein as SEQ#s 2167-4326 [see also WO00/66791].

The term 'protein of the invention' as used herein refers to a protein comprising:

- (a) one of sequences SEQ#s 1-4326; or
- 5 (b) a sequence having sequence identity to one of SEQ#s 1-4326; or
- (c) a fragment of one of SEQ#s 1-4326.

The degree of 'sequence identity' referred to in (b) is preferably greater than 50% (*eg.* 60%, 70%, 80%, 90%, 95%, 99% or more). This includes mutants and allelic variants [*e.g.* see WO00/66741]. Identity is preferably determined by the Smith-Waterman homology search  
10 algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty*=12 and *gap extension penalty*=1. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence.

The 'fragment' referred to in (c) should comprise at least *n* consecutive amino acids from  
15 one of SEQ#s 1-4326 and, depending on the particular sequence, *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or more). Preferably the fragment comprises an epitope from one of SEQ#s 1-4326. Preferred fragments are those disclosed in WO00/71574 and WO01/04316.

Preferred proteins of the invention are found in *N.meningitidis* serogroup B.

20 Preferred proteins for use according to the invention are those of serogroup B *N.meningitidis* strain 2996 or strain 394/98 (a New Zealand strain). Unless otherwise stated, proteins mentioned herein are from *N.meningitidis* strain 2996. It will be appreciated, however, that the invention is not in general limited by strain. References to a particular protein (*e.g.* '287', '919' *etc.*) may be taken to include that protein from any strain.

## 25 ***Non-fusion expression***

In a first approach to heterologous expression, no fusion partner is used, and the native leader peptide (if present) is used. This will typically prevent any 'interference' from fusion partners and may alter cellular localisation and/or post-translational modification and/or folding in the heterologous host.

Thus the invention provides a method for the heterologous expression of a protein of the invention, in which (a) no fusion partner is used, and (b) the protein's native leader peptide (if present) is used.

5 The method will typically involve the step of preparing an vector for expressing a protein of the invention, such that the first expressed amino acid is the first amino acid (methionine) of said protein, and last expressed amino acid is the last amino acid of said protein (*i.e.* the codon preceding the native STOP codon).

This approach is preferably used for the expression of the following proteins using the native leader peptide: 111, 149, 206, 225-1, 235, 247-1, 274, 283, 286, 292, 401, 406, 502-1, 503,  
10 519-1, 525-1, 552, 556, 557, 570, 576-1, 580, 583, 664, 759, 907, 913, 920-1, 936-1, 953, 961, 983, 989, Orf4, Orf7-1, Orf9-1, Orf23, Orf25, Orf37, Orf38, Orf40, Orf40.1, Orf40.2, Orf72-1, Orf76-1, Orf85-2, Orf91, Orf97-1, Orf119, Orf143.1, NMB0109 and NMB2050. The suffix 'L' used herein in the name of a protein indicates expression in this manner using the native leader peptide.

15 Proteins which are preferably expressed using this approach using no fusion partner and which have no native leader peptide include: 008, 105, 117-1, 121-1, 122-1, 128-1, 148, 216, 243, 308, 593, 652, 726, 926, 982, Orf83-1 and Orf143-1.

Advantageously, it is used for the expression of ORF25 or ORF40, resulting in a protein which induces better anti-bactericidal antibodies than GST- or His-fusions.

20 This approach is particularly suited for expressing lipoproteins.

#### ***Leader-peptide substitution***

In a second approach to heterologous expression, the native leader peptide of a protein of the invention is replaced by that of a different protein. In addition, it is preferred that no fusion partner is used. Whilst using a protein's own leader peptide in heterologous hosts can often  
25 localise the protein to its 'natural' cellular location, in some cases the leader sequence is not efficiently recognised by the heterologous host. In such cases, a leader peptide known to drive protein targeting efficiently can be used instead.

Thus the invention provides a method for the heterologous expression of a protein of the invention, in which (a) the protein's leader peptide is replaced by the leader peptide from a  
30 different protein and, optionally, (b) no fusion partner is used.

The method will typically involve the steps of: obtaining nucleic acid encoding a protein of the invention; manipulating said nucleic acid to remove nucleotides that encode the protein's leader peptide and to introduce nucleotides that encode a different protein's leader peptide. The resulting nucleic acid may be inserted into an expression vector, or may already be part of an expression vector. The expressed protein will consist of the replacement leader peptide at the N-terminus, followed by the protein of the invention minus its leader peptide.

The leader peptide is preferably from another protein of the invention (e.g. one of SEQ#s 1-4326), but may also be from an *E.coli* protein (e.g. the OmpA leader peptide) or an *Erwinia carotovora* protein (e.g. the PelB leader peptide), for instance.

10 A particularly useful replacement leader peptide is that of ORF4. This leader is able to direct lipidation in *E.coli*, improving cellular localisation, and is particularly useful for the expression of proteins 287, 919 and  $\Delta$ G287. The leader peptide and N-terminal domains of 961 are also particularly useful.

Another useful replacement leader peptide is that of *E.coli* OmpA. This leader is able to direct membrane localisation of *E.coli*. It is particularly advantageous for the expression of ORF1, resulting in a protein which induces better anti-bactericidal antibodies than both fusions and protein expressed from its own leader peptide.

Another useful replacement leader peptide is MKKYLFSAA. This can direct secretion into culture medium, and is extremely short and active. The use of this leader peptide is not restricted to the expression of Neisserial proteins – it may be used to direct the expression of any protein (particularly bacterial proteins).

#### ***Leader-peptide deletion***

In a third approach to heterologous expression, the native leader peptide of a protein of the invention is deleted. In addition, it is preferred that no fusion partner is used.

25 Thus the invention provides a method for the heterologous expression of a protein of the invention, in which (a) the protein's leader peptide is deleted and, optionally, (b) no fusion partner is used.

The method will typically involve the steps of: obtaining nucleic acid encoding a protein of the invention; manipulating said nucleic acid to remove nucleotides that encode the protein's leader peptide. The resulting nucleic acid may be inserted into an expression vector, or may

already be part of an expression vector. The first amino acid of the expressed protein will be that of the mature native protein.

This method can increase the levels of expression. For protein 919, for example, expression levels in *E.coli* are much higher when the leader peptide is deleted. Increased expression  
5 may be due to altered localisation in the absence of the leader peptide.

The method is preferably used for the expression of 919, ORF46, 961, 050-1, 760 and 287.

### ***Domain-based expression***

In a fourth approach to heterologous expression, the protein is expressed as domains. This may be used in association with fusion systems (*e.g.* GST or His-tag fusions).

10 Thus the invention provides a method for the heterologous expression of a protein of the invention, in which (a) at least one domain in the protein is deleted and, optionally, (b) no fusion partner is used.

The method will typically involve the steps of: obtaining nucleic acid encoding a protein of the invention; manipulating said nucleic acid to remove at least one domain from within the  
15 protein. The resulting nucleic acid may be inserted into an expression vector, or may already be part of an expression vector. Where no fusion partners are used, the first amino acid of the expressed protein will be that of a domain of the protein.

A protein is typically divided into notional domains by aligning it with known sequences in databases and then determining regions of the protein which show different alignment  
20 patterns from each other.

The method is preferably used for the expression of protein 287. This protein can be notionally split into three domains, referred to as A B & C (see Figure 5). Domain B aligns strongly with IgA proteases, domain C aligns strongly with transferrin-binding proteins, and domain A shows no strong alignment with database sequences. An alignment of  
25 polymorphic forms of 287 is disclosed in WO00/66741.

Once a protein has been divided into domains, these can be (a) expressed singly (b) deleted from with the protein *e.g.* protein ABCD → ABD, ACD, BCD *etc.* or (c) rearranged *e.g.* protein ABC → ACB, CAB *etc.* These three strategies can be combined with fusion partners is desired.

ORF46 has also been notionally split into two domains – a first domain (amino acids 1-433) which is well-conserved between species and serogroups, and a second domain (amino acids 433-608) which is not well-conserved. The second domain is preferably deleted. An alignment of polymorphic forms of ORF46 is disclosed in WO00/66741.

- 5 Protein 564 has also been split into domains (Figure 8), as have protein 961 (Figure 12) and protein 502 (amino acids 28-167 of the MC58 protein).

### ***Hybrid proteins***

- 10 In a fifth approach to heterologous expression, two or more (*e.g.* 3, 4, 5, 6 or more) proteins of the invention are expressed as a single hybrid protein. It is preferred that no non-Neisserial fusion partner (*e.g.* GST or poly-His) is used.

This offers two advantages. Firstly, a protein that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem. Secondly, commercial manufacture is simplified – only one expression and purification need be employed in order to produce two separately-useful proteins.

- 15 Thus the invention provides a method for the simultaneous heterologous expression of two or more proteins of the invention, in which said two or more proteins of the invention are fused (*i.e.* they are translated as a single polypeptide chain).

- 20 The method will typically involve the steps of: obtaining a first nucleic acid encoding a first protein of the invention; obtaining a second nucleic acid encoding a second protein of the invention; ligating the first and second nucleic acids. The resulting nucleic acid may be inserted into an expression vector, or may already be part of an expression vector.

Preferably, the constituent proteins in a hybrid protein according to the invention will be from the same strain.

- 25 The fused proteins in the hybrid may be joined directly, or may be joined via a linker peptide *e.g.* via a poly-glycine linker (*i.e.*  $G_n$  where  $n = 3, 4, 5, 6, 7, 8, 9, 10$  or more) or via a short peptide sequence which facilitates cloning. It is evidently preferred not to join a  $\Delta G$  protein to the C-terminus of a poly-glycine linker.

The fused proteins may lack native leader peptides or may include the leader peptide sequence of the N-terminal fusion partner.

The method is well suited to the expression of proteins orf1, orf4, orf25, orf40, Orf46/46.1, orf83, 233, 287, 292L, 564, 687, 741, 907, 919, 953, 961 and 983.

The 42 hybrids indicated by 'X' in the following table of form  $\text{NH}_2\text{-A-B-COOH}$  are preferred:

↓A B→	ORF46.1	287	741	919	953	961	983
ORF46.1		X	X	X	X	X	X
287	X		X	X	X	X	X
741	X	X		X	X	X	X
919	X	X	X		X	X	X
953	X	X	X	X		X	X
961	X	X	X	X	X		X
983	X	X	X	X	X	X	

- 5 Preferred proteins to be expressed as hybrids are thus ORF46.1, 287, 741, 919, 953, 961 and 983. These may be used in their essentially full-length form, or poly-glycine deletions ( $\Delta\text{G}$ ) forms may be used (*e.g.*  $\Delta\text{G-287}$ ,  $\Delta\text{GTbp2}$ ,  $\Delta\text{G741}$ ,  $\Delta\text{G983}$  *etc.*), or truncated forms may be used (*e.g.*  $\Delta\text{1-287}$ ,  $\Delta\text{2-287}$  *etc.*), or domain-deleted versions may be used (*e.g.* 287B, 287C, 287BC, ORF46<sub>1-433</sub>, ORF46<sub>433-608</sub>, ORF46, 961c *etc.*).
- 10 Particularly preferred are: (a) a hybrid protein comprising 919 and 287; (b) a hybrid protein comprising 953 and 287; (c) a hybrid protein comprising 287 and ORF46.1; (d) a hybrid protein comprising ORF1 and ORF46.1; (e) a hybrid protein comprising 919 and ORF46.1; (f) a hybrid protein comprising ORF46.1 and 919; (g) a hybrid protein comprising ORF46.1, 287 and 919; (h) a hybrid protein comprising 919 and 519; and (i) a hybrid protein
- 15 comprising ORF97 and 225. Further embodiments are shown in Figure 14.

Where 287 is used, it is preferably at the C-terminal end of a hybrid; if it is to be used at the N-terminus, if is preferred to use a  $\Delta\text{G}$  form of 287 is used (*e.g.* as the N-terminus of a hybrid with ORF46.1, 919, 953 or 961).

Where 287 is used, this is preferably from strain 2996 or from strain 394/98.

- 20 Where 961 is used, this is preferably at the N-terminus. Domain forms of 961 may be used.

Alignments of polymorphic forms of ORF46, 287, 919 and 953 are disclosed in WO00/66741. Any of these polymorphs can be used according to the present invention.

### ***Temperature***

In a sixth approach to heterologous expression, proteins of the invention are expressed at a low temperature.

Expressed Neisserial proteins (*e.g.* 919) may be toxic to *E.coli*, which can be avoided by  
5 expressing the toxic protein at a temperature at which its toxic activity is not manifested.

Thus the present invention provides a method for the heterologous expression of a protein of the invention, in which expression of a protein of the invention is carried out at a temperature at which a toxic activity of the protein is not manifested.

A preferred temperature is around 30°C. This is particularly suited to the expression of 919.

### 10 ***Mutations***

As discussed above, expressed Neisserial proteins may be toxic to *E.coli*. This toxicity can be avoided by mutating the protein to reduce or eliminate the toxic activity. In particular, mutations to reduce or eliminate toxic enzymatic activity can be used, preferably using site-directed mutagenesis.

15 In a seventh approach to heterologous expression, therefore, an expressed protein is mutated to reduce or eliminate toxic activity.

Thus the invention provides a method for the heterologous expression of a protein of the invention, in which protein is mutated to reduce or eliminate toxic activity.

The method is preferably used for the expression of protein 907, 919 or 922. A preferred  
20 mutation in 907 is at Glu-117 (*e.g.* Glu→Gly); preferred mutations in 919 are at Glu-255 (*e.g.* Glu→Gly) and/or Glu-323 (*e.g.* Glu→Gly); preferred mutations in 922 are at Glu-164 (*e.g.* Glu→Gly), Ser-213 (*e.g.* Ser→Gly) and/or Asn-348 (*e.g.* Asn→Gly).

### ***Alternative vectors***

In a eighth approach to heterologous expression, an alternative vector used to express the  
25 protein. This may be to improve expression yields, for instance, or to utilise plasmids that are already approved for GMP use.

Thus the invention provides a method for the heterologous expression of a protein of the invention, in which an alternative vector is used. The alternative vector is preferably pSM214, with no fusion partners. Leader peptides may or may not be included.

This approach is particularly useful for protein 953. Expression and localisation of 953 with its native leader peptide expressed from pSM214 is much better than from the pET vector.

pSM214 may also be used with:  $\Delta$ G287,  $\Delta$ 2-287,  $\Delta$ 3-287,  $\Delta$ 4-287, Orf46.1, 961L, 961, 961(MC58), 961c, 961c-L, 919, 953 and  $\Delta$ G287-Orf46.1.

- 5 Another suitable vector is pET-24b (Novagen; uses kanamycin resistance), again using no fusion partners. pET-24b is preferred for use with:  $\Delta$ G287K,  $\Delta$ 2-287K,  $\Delta$ 3-287K,  $\Delta$ 4-287K, Orf46.1-K, Orf46A-K, 961-K (MC58), 961a-K, 961b-K, 961c-K, 961c-L-K, 961d-K,  $\Delta$ G287-919-K,  $\Delta$ G287-Orf46.1-K and  $\Delta$ G287-961-K.

### ***Multimeric form***

- 10 In a ninth approach to heterologous expression, a protein is expressed or purified such that it adopts a particular multimeric form.

This approach is particularly suited to protein 953. Purification of one particular multimeric form of 953 (the monomeric form) gives a protein with greater bactericidal activity than other forms (the dimeric form).

- 15 Proteins 287 and 919 may be purified in dimeric forms.

Protein 961 may be purified in a 180kDa oligomeric form (*e.g.* a tetramer).

### ***Lipidation***

In a tenth approach to heterologous expression, a protein is expressed as a lipidated protein.

- 20 Thus the invention provides a method for the heterologous expression of a protein of the invention, in which the protein is expressed as a lipidated protein.

This is particularly useful for the expression of 919, 287, ORF4, 406, 576-1, and ORF25. Polymorphic forms of 919, 287 and ORF4 are disclosed in WO00/66741.

The method will typically involve the use of an appropriate leader peptide without using an N-terminal fusion partner.

- 25 ***C-terminal deletions***

In an eleventh approach to heterologous expression, the C-terminus of a protein of the invention is mutated. In addition, it is preferred that no fusion partner is used.

Thus the invention provides a method for the heterologous expression of a protein of the invention, in which (a) the protein's C-terminus region is mutated and, optionally, (b) no fusion partner is used.

5 The method will typically involve the steps of: obtaining nucleic acid encoding a protein of the invention; manipulating said nucleic acid to mutate nucleotides that encode the protein's C-terminus portion. The resulting nucleic acid may be inserted into an expression vector, or may already be part of an expression vector. The first amino acid of the expressed protein will be that of the mature native protein.

The mutation may be a substitution, insertion or, preferably, a deletion.

10 This method can increase the levels of expression, particularly for proteins 730, ORF29 and ORF46. For protein 730, a C-terminus region of around 65 to around 214 amino acids may be deleted; for ORF46, the C-terminus region of around 175 amino acids may be deleted; for ORF29, the C-terminus may be deleted to leave around 230-370 N-terminal amino acids.

#### ***Leader peptide mutation***

15 In a twelfth approach to heterologous expression, the leader peptide of the protein is mutated. This is particularly useful for the expression of protein 919.

Thus the invention provides a method for the heterologous expression of a protein of the invention, in which the protein's leader peptide is mutated.

20 The method will typically involve the steps of: obtaining nucleic acid encoding a protein of the invention; and manipulating said nucleic acid to mutate nucleotides within the leader peptide. The resulting nucleic acid may be inserted into an expression vector, or may already be part of an expression vector.

#### ***Poly-glycine deletion***

25 In a thirteenth approach to heterologous expression, poly-glycine stretches in wild-type sequences are mutated. This enhances protein expression.

The poly-glycine stretch has the sequence (Gly)<sub>n</sub>, where  $n \geq 4$  (e.g. 5, 6, 7, 8, 9 or more). This stretch is mutated to disrupt or remove the (Gly)<sub>n</sub>. This may be by deletion (e.g. CGGGGS → CGGGS, CGGS, CGS or CS), by substitution (e.g. CGGGGS → CGXGGS, CGXXGS, CGXGXS etc.), and/or by insertion (e.g. CGGGGS → CGGXGGS, CGXGGGS, etc.).

This approach is not restricted to Neisserial proteins – it may be used for any protein (particularly bacterial proteins) to enhance heterologous expression. For Neisserial proteins, however, it is particularly suitable for expressing 287, 741, 983 and Tbp2. An alignment of polymorphic forms of 287 is disclosed in WO00/66741.

- 5 Thus the invention provides a method for the heterologous expression of a protein of the invention, in which (a) a poly-glycine stretch within the protein is mutated.

The method will typically involve the steps of: obtaining nucleic acid encoding a protein of the invention; and manipulating said nucleic acid to mutate nucleotides that encode a poly-glycine stretch within the protein sequence. The resulting nucleic acid may be inserted into  
10 an expression vector, or may already be part of an expression vector.

Conversely, the opposite approach (*i.e.* introduction of poly-glycine stretches) can be used to suppress or diminish expression of a given heterologous protein.

#### ***Heterologous host***

Whilst expression of the proteins of the invention may take place in the native host (*i.e.* the organism in which the protein is expressed in nature), the present invention utilises a  
15 heterologous host. The heterologous host may be prokaryotic or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi*, *Salmonella typhimurium*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Neisseria lactamica*, *Neisseria cinerea*, *Mycobacteria* (*e.g.* *M.tuberculosis*), yeast *etc.*

#### ***Vectors etc.***

As well as the methods described above, the invention provides (a) nucleic acid and vectors useful in these methods (b) host cells containing said vectors (c) proteins expressed or expressable by the methods (d) compositions comprising these proteins, which may be suitable as vaccines, for instance, or as diagnostic reagents, or as immunogenic compositions  
25 (e) these compositions for use as medicaments (*e.g.* as vaccines) or as diagnostic reagents (f) the use of these compositions in the manufacture of (1) a medicament for treating or preventing infection due to Neisserial bacteria (2) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria, and/or (3) a reagent which can raise antibodies against Neisserial bacteria and (g) a method of treating a

patient, comprising administering to the patient a therapeutically effective amount of these compositions.

### ***Sequences***

5 The invention also provides a protein or a nucleic acid having any of the sequences set out in the following examples. It also provides proteins and nucleic acid having sequence identity to these. As described above, the degree of 'sequence identity' is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more).

Furthermore, the invention provides nucleic acid which can hybridise to the nucleic acid disclosed in the examples, preferably under "high stringency" conditions (eg. 65°C in a  
10 0.1xSSC, 0.5% SDS solution).

The invention also provides nucleic acid encoding proteins according to the invention.

It should also be appreciated that the invention provides nucleic acid comprising sequences complementary to those described above (eg. for antisense or probing purposes).

15 Nucleic acid according to the invention can, of course, be prepared in many ways (eg. by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc.*) and can take various forms (eg. single stranded, double stranded, vectors, probes *etc.*).

In addition, the term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc.*

### **BRIEF DESCRIPTION OF DRAWINGS**

20 Figures 1 and 2 show constructs used to express proteins using heterologous leader peptides.

Figure 3 shows expression data for ORF1, and Figure 4 shows similar data for protein 961.

Figure 5 shows domains of protein 287, and Figures 6 & 7 show deletions within domain A.

Figure 8 shows domains of protein 564.

25 Figure 9 shows the *PhoC* reporter gene driven by the 919 leader peptide, and Figure 10 shows the results obtained using mutants of the leader peptide.

Figure 11 shows insertion mutants of protein 730 (A: 730-C1; B: 730-C2).

Figure 12 shows domains of protein 961.

Figure 13 shows SDS-PAGE of ΔG proteins. Dots show the main recombinant product.

Figure 14 shows 26 hybrid proteins according to the invention.

## MODES FOR CARRYING OUT THE INVENTION

### Example 1 – 919 and its leader peptide

- 5 Protein 919 from *N.meningitidis* (serogroup B, strain 2996) has the following sequence:

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1  MKKYLFRAAL YGIAAAILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
51  GTTVGGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
101 CAQAFQTPVH SFQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR
151 RTAQARFPIY GIPDDFISVP LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT
201 HTADLSRFPI TARTTAIKGR FEGRSFLPYH TRNQINGGAL DGKAPILGYA
251 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
301 KLGQTSMQGI KAYMRQNPQR LAEVLGQNPS YIFFRELAYS SNDGPGVAGL
351 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG
401 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P*
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- 15 The leader peptide is underlined.

The sequences of 919 from other strains can be found in Figures 7 and 18 of WO00/66741.

Example 2 of WO99/57280 discloses the expression of protein 919 as a His-fusion in *E.coli*.

The protein is a good surface-exposed immunogen.

Three alternative expression strategies were used for 919:

- 20 1) 919 without its leader peptide (and without the mature N-terminal cysteine) and without any fusion partner ('919<sup>untagged</sup>):

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1  QSKSIQTFP QPDTSVINGP DRPVGIPDPA GTTVGGGGAV YTVVPHLSLP
50  HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV CAQAFQTPVH SFQAKQFFER
100 YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR RTAQARFPIY GIPDDFISVP
25 150 LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT HTADLSRFPI TARTTAIKGR
200 FEGRSFLPYH TRNQINGGAL DGKAPILGYA EDPVELFFMH IQGSGRLKTP
250 SGKYIRIGYA DKNEHPYVSI GRYMADKGYL KLGQTSMQGI KAYMRQNPQR
300 LAEVLGQNPS YIFFRELAYS SNDGPGVAGL TPLMGEYAGA VDRHYITLGA
350 PLFVATAHPV TRKALNRLIM AQDTGSAIKG AVRVDYFWGY GDEAGELAGK
30 400 QKTTGYVWQL LPNGMKPEYR P*
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The leader peptide and cysteine were omitted by designing the 5'-end amplification primer downstream from the predicted leader sequence.

- 2) 919 with its own leader peptide but without any fusion partner ('919L'); and  
35 3) 919 with the leader peptide (MKTFFKTL<sup>S</sup>AAALALILAA) from ORF4 ('919L<sup>Orf4</sup>).

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1  MKTFFKTLS AAALALILAA CQSKSIQTFP QPDTSVINGP DRPVGIPDPA
50  GTTVGGGGAV YTVVPHLSLP HWAAQDFAKS LQSFRLGCAN LKNRQGWQDV
100 CAQAFQTPVH SFQAKQFFER YFTPWQVAGN GSLAGTVTGY YEPVLKGDDR
150 RTAQARFPIY GIPDDFISVP LPAGLRSGKA LVRIRQTGKN SGTIDNTGGT
40 200 HTADLSRFPI TARTTAIKGR FEGRSFLPYH TRNQINGGAL DGKAPILGYA
250 EDPVELFFMH IQGSGRLKTP SGKYIRIGYA DKNEHPYVSI GRYMADKGYL
300 KLGQTSMQGI KSYMQRNPQR LAEVLGQNPS YIFFRELAYS SNDGPGVAGL
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350 TPLMGEYAGA VDRHYITLGA PLFVATAHPV TRKALNRLIM AQDTGSAIKG  
 400 AVRVDYFWGY GDEAGELAGK QKTTGYVWQL LPNGMKPEYR P\*

5 To make this construct, the entire sequence encoding the ORF4 leader peptide was included in the 5'-primer as a tail (primer 919Lorf4 For). A *NheI* restriction site was generated by a double nucleotide change in the sequence coding for the ORF4 leader (no amino acid changes), to allow different genes to be fused to the ORF4 leader peptide sequence. A stop codon was included in all the 3'-end primer sequences.

All three forms of the protein were expressed and could be purified.

10 The '919L' and '919Lorf4' expression products were both lipidated, as shown by the incorporation of [<sup>3</sup>H]-palmitate label. 919<sup>untagged</sup> did not incorporate the <sup>3</sup>H label and was located intracellularly.

919Lorf4 could be purified more easily than 919L. It was purified and used to immunise mice. The resulting sera gave excellent results in FACS and ELISA tests, and also in the  
 15 bactericidal assay. The lipoprotein was shown to be localised in the outer membrane.

919<sup>untagged</sup> gave excellent ELISA titres and high serum bactericidal activity. FACS confirmed its cell surface location.

#### *Example 2 – 919 and expression temperature*

Growth of *E.coli* expressing the 919Lorf4 protein at 37°C resulted in lysis of the bacteria. In  
 20 order to overcome this problem, the recombinant bacteria were grown at 30°C. Lysis was prevented without preventing expression.

#### *Example 3 – mutation of 907, 919 and 922*

It was hypothesised that proteins 907, 919 and 922 are murein hydrolases, and more particularly lytic transglycosylases. Murein hydrolases are located on the outer membrane  
 25 and participate in the degradation of peptidoglycan.

The purified proteins 919<sup>untagged</sup>, 919Lorf4, 919-His (*i.e.* with a C-terminus His-tag) and 922-His were thus tested for murein hydrolase activity [Ursinus & Holtje (1994) *J.Bact.* 176:338-343]. Two different assays were used, one determining the degradation of insoluble murein sacculus into soluble muropeptides and the other measuring breakdown of  
 30 poly(MurNAc-GlcNAc)<sub>n>30</sub> glycan strands.

The first assay uses murein sacculi radiolabelled with meso-2,6-diamino-3,4,5- $^3\text{H}$ pimelic acid as substrate. Enzyme (3–10  $\mu\text{g}$  total) was incubated for 45 minutes at 37°C in a total volume of 100 $\mu\text{l}$  comprising 10mM Tris-maleate (pH 5.5), 10mM  $\text{MgCl}_2$ , 0.2% v/v Triton X-100 and  $^3\text{H}$ A<sub>2</sub>pm labelled murein sacculi (about 10000cpm). The assay mixture was  
5 placed on ice for 15 minutes with 100  $\mu\text{l}$  of 1% w/v N-acetyl-N,N,N-trimethylammonium for 15 minutes and precipitated material pelleted by centrifugation at 10000g for 15 minutes. The radioactivity in the supernatant was measured by liquid scintillation counting. *E.coli* soluble lytic transglycosylase Slt70 was used as a positive control for the assay; the negative control comprised the above assay solution without enzyme.

10 All proteins except 919-His gave positive results in the first assay.

The second assay monitors the hydrolysis of poly(MurNAc-GlcNAc)glycan strands. Purified strands, poly(MurNAc-GlcNAc)<sub>n>30</sub> labelled with N-acetyl-D-1- $^3\text{H}$ glucosamine were incubated with 3 $\mu\text{g}$  of 919L in 10 mM Tris-maleate (pH 5.5), 10 mM  $\text{MgCl}_2$  and 0.2% v/v Triton X-100 for 30 min at 37°C. The reaction was stopped by boiling for 5 minutes and the  
15 pH of the sample adjusted to about 3.5 by addition of 10 $\mu\text{l}$  of 20% v/v phosphoric acid. Substrate and product were separated by reversed phase HPLC on a Nucleosil 300 C<sub>18</sub> column as described by Harz *et. al.* [*Anal. Biochem.* (1990) 190:120-128]. The *E.coli* lytic transglycosylase Mlt A was used as a positive control in the assay. The negative control was performed in the absence of enzyme.

20 By this assay, the ability of 919Lorf4 to hydrolyse isolated glycan strands was demonstrated when anhydrodisaccharide subunits were separated from the oligosaccharide by HPLC.

Protein 919Lorf4 was chosen for kinetic analyses. The activity of 919Lorf4 was enhanced 3.7-fold by the addition of 0.2% v/v Triton X-100 in the assay buffer. The presence of Triton X-100 had no effect on the activity of 919<sup>untagged</sup>. The effect of pH on enzyme activity was  
25 determined in Tris-Maleate buffer over a range of 5.0 to 8.0. The optimal pH for the reaction was determined to be 5.5. Over the temperature range 18°C to 42°C, maximum activity was observed at 37°C. The effect of various ions on murein hydrolase activity was determined by performing the reaction in the presence of a variety of ions at a final concentration of 10mM. Maximum activity was found with  $\text{Mg}^{2+}$ , which stimulated activity 2.1-fold.  $\text{Mn}^{2+}$  and  $\text{Ca}^{2+}$   
30 also stimulated enzyme activity to a similar extent while the addition  $\text{Ni}^{2+}$  and EDTA had no significant effect. In contrast, both  $\text{Fe}^{2+}$  and  $\text{Zn}^{2+}$  significantly inhibited enzyme activity.

The structures of the reaction products resulting from the digestion of unlabelled *E.coli* murein sacculus were analysed by reversed-phase HPLC as described by Glauner [*Anal. Biochem.* (1988) 172:451-464]. Murein sacculi digested with the muramidase Cellosyl were used to calibrate and standardise the Hypersil ODS column. The major reaction products  
5 were 1,6 anhydrodisaccharide tetra and tri peptides, demonstrating the formation of 1,6 anhydromuraminic acid intramolecular bond.

These results demonstrate experimentally that 919 is a murein hydrolase and in particular a member of the lytic transglycosylase family of enzymes. Furthermore the ability of 922-His to hydrolyse murein sacculi suggests this protein is also a lytic transglycosylase.

10 This activity may help to explain the toxic effects of 919 when expressed in *E.coli*.

In order to eliminate the enzymatic activity, rational mutagenesis was used. 907, 919 and 922 show fairly low homology to three membrane-bound lipidated murein lytic transglycosylases from *E.coli*:

- 919 (441aa) is 27.3% identical over 440aa overlap to *E.coli* MLTA (P46885);  
15 922 (369aa) is 38.7% identical over 310aa overlap to *E.coli* MLTB (P41052); and  
907-2 (207aa) is 26.8% identical over 149aa overlap to *E.coli* MLTC (P52066).

907-2 also shares homology with *E.coli* MLTD (P23931) and Slt70 (P03810), a soluble lytic transglycosylase that is located in the periplasmic space. No significant sequence homology can be detected among 919, 922 and 907-2, and the same is true among the corresponding  
20 MLTA, MLTB and MLTC proteins.

Crystal structures are available for Slt70 [1QTEA; 1QTEB; Thunnissen *et al.* (1995) *Biochemistry* 34:12729-12737] and for Slt35 [1LTM; 1QUS; 1QUT; van Asselt *et al.* (1999) *Structure Fold Des* 7:1167-80] which is a soluble form of the 40kDa MLTB.

The catalytic residue (a glutamic acid) has been identified for both Slt70 and MLTB.

25 In the case of Slt70, mutagenesis studies have demonstrated that even a conservative substitution of the catalytic Glu505 with a glutamine (Gln) causes the complete loss of enzymatic activity. Although Slt35 has no obvious sequence similarity to Slt70, their catalytic domains shows a surprising similarity. The corresponding catalytic residue in MLTB is Glu162.

Another residue which is believed to play an important role in the correct folding of the enzymatic cleft is a well-conserved glycine (Gly) downstream of the glutamic acid. Recently, Terrak *et al.* [*Mol.Microbiol.* (1999) 34:350-64] have suggested the presence of another important residue which is an aromatic amino acid located around 70-75 residues downstream of the catalytic glutamic acid.

Sequence alignment of Slt70 with 907-2 and of MLTB with 922 were performed in order to identify the corresponding catalytic residues in the MenB antigens.

The two alignments in the region of the catalytic domain are reported below:

#### 907-2/Slt70:

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10      90      100      110      ▼120      130      140
      907-2.pep  ERRRLVNIQYESSRAG--LDTQIVLGLIEVESAFRQYAIISGVGARGLMQVMPFWKNYIG
      || | | : : : : : : : : : : : : : : : : : : : : : : : : : : : :
      slty_ecoli  ERFPLAYNDLFKRYTSGKEIPQSYAMAIARQESAWNPKVKSPVGASGLMQIMPGTATHTV
      480      490      500      ▲ 510      520      530
                        GLU505

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#### 922/MLTB

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20      150      160      ▼ 170      180      190      200
      922.pep    VAQKYGVPAELIVAVIGIETNYGKNTGSFRVADALATLGFDYPRRAGFFQKELVELLKLA
      : | ||| | : || : || : || : || : || : || : || : || : || : || : || : ||
      mltb_ecoli  AWQVYGVPPETIIVGIIGVETRWGRVMGKTRILDALATLSFNYPRAEYFSGELETFLMA
      150      160      ▲ 170      180      190      200
                        GLU162

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25      210      220      230      240      250      260
      922.pep    KEEGGDVFAFKGSYAGAMGMPQFMPSSYRKWAVDYDGDGHRDIWGNVGDVAASVANYMKQ
      : : | | : : ||| : ||| : ||| : ||| : ||| : ||| : ||| : ||| : ||| : |||
      mltb_ecoli  RDEQDDPLNLKGSFAGAMGYQFMPSSYKQYAVDFSGDGHINLWDPV-DAIGSVANYFKA
      210      220      230      240      250      260

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From these alignments, it results that the corresponding catalytic glutamate in 907-2 is Glu117, whereas in 922 is Glu164. Both antigens also share downstream glycines that could have a structural role in the folding of the enzymatic cleft (in bold), and 922 has a conserved aromatic residue around 70aa downstream (in bold).

In the case of protein 919, no 3D structure is available for its *E.coli* homologue MLTA, and nothing is known about a possible catalytic residue. Nevertheless, three amino acids in 919 are predicted as catalytic residues by alignment with MLTA:

#### 919/MLTA

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40      240      250      ▼ 260 □ □ 270 □      280      290
      919.pep    ALDGKAPILGYAEDPVELFFMHIQSGRLKTPSGKYIRI-GYADKNEHPVVSIGRYMADK
      || : | || : || : || : || : || : || : || : || : || : || : || : || : ||
      mlta_ecoli.p ALSDKY-ILAYSNSLMDNFIMDVQSGGYIDFGDGSPLNFFSYAGKNGHAYRSIGKVLIDR
      170      180      190      200      210

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-18-

	300	310	320 ▼	330 □ □	340	0350	0
919.pep	GYLKLQTS	MOGIKSYM	RQNPQ-RL	AEVLGQNP	SYIFFREL	AGSSNDGPV-	GALGTPLMG
	:	:	: : : : : :	:	:	: :	:
5 mlta_ecoli.p	GEVKKEDMS	MQAIRHWG	ETHSEAEV	RELLEQN	PSFVFFK	PQSFA----	PVKGASAVPLVG
	220	230	240	250	260		270
	360 ▼	o	380	390	400	00410	
919.pep	EYAGAVDR	HYITLGAP	LFVATAHP	VTTRKALN	-----	RLIMAQDTG	SAIKGAVRV
	: :	:	: : : : :	: :	:	:	:   :
10 mlta_ecoli.p	RASVASDR	SIIPPGTT	LLAEVPL	LDNNGKF	NGQYELRL	MVALDVGG	AIKQGQ-HF
	280	290	300	310	320		330
	420	o					
919.pep	GDEAGEL	AGKQKT	TGYVWQ	LLP			
		:	:				
15 mlta_ecoli.p	GPEAGH	RAGWYN	HYGRVW	VVLKT			
	340		350				

The three possible catalytic residues are shown by the symbol ▼:

- 20 1) Glu255 (Asp in MLTA), followed by three conserved glycines (Gly263, Gly265 and Gly272) and three conserved aromatic residues located approximately 75-77 residues downstream. These downstream residues are shown by □.
- 2) Glu323 (conserved in MLTA), followed by 2 conserved glycines (Gly347 and Gly355) and two conserved aromatic residues located 84-85 residues downstream (Tyr406 or Phe407). These downstream residues are shown by ◇.
- 25 3) Asp362 (instead of the expected Glu), followed by one glycine (Gly 369) and a conserved aromatic residue (Trp428). These downstream residues are shown by o.

Alignments of polymorphic forms of 919 are disclosed in WO00/66741.

- Based on the prediction of catalytic residues, three mutants of the 919 and one mutant of
- 30 907, containing each a single amino acid substitution, have been generated. The glutamic acids in position 255 and 323 and the aspartic acids in position 362 of the 919 protein and the glutamic acid in position 117 of the 907 protein, were replaced with glycine residues using PCR-based SDM. To do this, internal primers containing a codon change from Glu or Asp to Gly were designed:

Primers	Sequences	Codon change
919-E255 for 919-E255 rev	CGAAGACCCCGTC <u>Ggt</u> CTTTTTTTTATG GTGCATAAAAAAAGacCGACGGGGTCT	GAA → Ggt
919-E323 for 919-E323 rev	AACGCCTCGCC <u>Ggt</u> GTTTTGGGTCA TTTGACCCAAAACacCGGCGAGGCG	GAA → Ggt
919-D362 for 919-D362 rev	TGCCGGCGCAGTC <u>Ggt</u> CGGCACTACA TAATGTAGTGCCGacCGACTGCGCCG	GAC → Ggt
907-E117 for 907-E117 rev	TGATTGAGGTG <u>Ggt</u> AGCGCGTTCCG GGCGGAACGCGCTacCCACCTCAAT	GAA → Ggt

Underlined nucleotides code for glycine; the mutated nucleotides are in lower case.

To generate the 919-E255, 919-E323 and 919-E362 mutants, PCR was performed using 20ng of the pET 919-Lorf4 DNA as template, and the following primer pairs:

- 1) Orf4L for / 919-E255 rev
- 2) 919-E255 for / 919L rev
- 3) Orf4L for / 919-E323 rev
- 4) 919-E323 for / 919L rev
- 5) Orf4L for / 919-D362 rev
- 6) 919-D362 for / 919L rev

- 10 The second round of PCR was performed using the product of PCR 1-2, 3-4 or 5-6 as template, and as forward and reverse primers the "Orf4L for" and "919L rev" respectively.

For the mutant 907-E117, PCR have been performed using 200ng of chromosomal DNA of the 2996 strain as template and the following primer pairs:

- 7) 907L for / 907-E117 rev
- 8) 907-E117 for / 907L rev

The second round of PCR was performed using the products of PCR 7 and 8 as templates and the oligos "907L for" and "907L rev" as primers.

- 20 The PCR fragments containing each mutation were processed following the standard procedure, digested with *Nde*I and *Xho*I restriction enzymes and cloned into pET-21b+ vector. The presence of each mutation was confirmed by sequence analysis.

Mutation of Glu117 to Gly in 907 is carried out similarly, as is mutation of residues Glu164, Ser213 and Asn348 in 922.

The E255G mutant of 919 shows a 50% reduction in activity; the E323G mutant shows a 70% reduction in activity; the E362G mutant shows no reduction in activity.

**Example 4 – multimeric form**

287-GST, 919<sup>untagged</sup> and 953-His were subjected to gel filtration for analysis of quaternary structure or preparative purposes. The molecular weight of the native proteins was estimated using either FPLC Superose 12 (H/R 10/30) or Superdex 75 gel filtration columns (Pharmacia). The buffers used for chromatography for 287, 919 and 953 were 50 mM Tris-HCl (pH 8.0), 20 mM Bicine (pH 8.5) and 50 mM Bicine (pH 8.0), respectively.

Additionally each buffer contained 150-200 mM NaCl and 10% v/v glycerol. Proteins were dialysed against the appropriate buffer and applied in a volume of 200µl. Gel filtration was performed with a flow rate of 0.5 – 2.0 ml/min and the eluate monitored at 280nm. Fractions were collected and analysed by SDS-PAGE. Blue dextran 2000 and the molecular weight standards ribonuclease A, chymotrypsin A ovalbumin, albumin (Pharmacia) were used to calibrate the column. The molecular weight of the sample was estimated from a calibration curve of  $K_{av}$  vs.  $\log M_r$  of the standards. Before gel filtration, 287-GST was digested with thrombin to cleave the GST moiety.

The estimated molecular weights for 287, 919 and 953-His were 73 kDa, 47 kDa and 43 kDa respectively. These results suggest 919 is monomeric while both 287 and 953 are principally dimeric in their nature. In the case of 953-His, two peaks were observed during gel filtration. The major peak (80%) represented a dimeric conformation of 953 while the minor peak (20%) had the expected size of a monomer. The monomeric form of 953 was found to have greater bactericidal activity than the dimer.

**Example 5 – pSM214 and pET-24b vectors**

953 protein with its native leader peptide and no fusion partners was expressed from the pET vector and also from pSM214 [Velati Bellini *et al.* (1991) *J. Biotechnol.* 18, 177-192].

The 953 sequence was cloned as a full-length gene into pSM214 using the *E. coli* MM294-1 strain as a host. To do this, the entire DNA sequence of the 953 gene (from ATG to the STOP codon) was amplified by PCR using the following primers:

25	953L for/2 CCGGAATTCTTATGAAAAAATCATCTTCGCCGC	Eco RI
30	953L rev/2 GCCCAAGCTTTTATTGTTTGGCTGCCTCGATT	Hind III

which contain *EcoRI* and *HindIII* restriction sites, respectively. The amplified fragment was digested with *EcoRI* and *HindIII* and ligated with the pSM214 vector digested with the same two enzymes. The ligated plasmid was transformed into *E.coli* MM294-1 cells (by incubation in ice for 65 minutes at 37° C) and bacterial cells plated on LB agar containing 20µg/ml of chloramphenicol.

Recombinant colonies were grown over-night at 37°C in 4 ml of LB broth containing 20 µg/ml of chloramphenicol; bacterial cells were centrifuged and plasmid DNA extracted as and analysed by restriction with *EcoRI* and *HindIII*. To analyse the ability of the recombinant colonies to express the protein, they were inoculated in LB broth containing 20µg/ml of chloramphenicol and let to grown for 16 hours at 37°C. Bacterial cells were centrifuged and resuspended in PBS. Expression of the protein was analysed by SDS-PAGE and Coomassie Blue staining.

Expression levels were unexpectedly high from the pSM214 plasmid.

Oligos used to clone sequences into pSM-214 vectors were as follows:

<b>ΔG287</b> (pSM-214)	Fwd	CCGGAATTCCTTATG-TCGCCCCGATGTAAATCGGCGGA	EcoRI
	Rev	GCCCAAGCTT-TCAATCCTGCTCTTTTTTGCCG	HindIII
<b>Δ2 287</b> (pSM-214)	Fwd	CCGGAATTCCTTATG-AGCCAAGATATGGCGGCAGT	EcoRI
	Rev	GCCCAAGCTT-TCAATCCTGCTCTTTTTTGCCG	HindIII
<b>Δ3 287</b> (pSM-214)	Fwd	CCGGAATTCCTTATG-TCCGCCGAATCCGCAAATCA	EcoRI
	Rev	GCCCAAGCTT-TCAATCCTGCTCTTTTTTGCCG	HindIII
<b>Δ4 287</b> (pSM-214)	Fwd	CCGGAATTCCTTATG-GGAAGGGTTGATTTGGCTAATG	EcoRI
	Rev	GCCCAAGCTT-TCAATCCTGCTCTTTTTTGCCG	HindIII
<b>Orf46.1</b> (pSM-214)	Fwd	CCGGAATTCCTTATG-TCAGATTGGCAAACGATTCTT	EcoRI
	Rev	GCCCAAGCTT-TTACGTATCATATTTACGTGCTTC	HindIII
<b>ΔG287-Orf46.1</b> (pSM-214)	Fwd	CCGGAATTCCTTATG-TCGCCCCGATGTAAATCGGCGGA	EcoRI
	Rev	GCCCAAGCTT-TTACGTATCATATTTACGTGCTTC	HindIII
<b>919</b> (pSM-214)	Fwd	CCGGAATTCCTTATG-CAAAGCAAGAGCATCCAAACCT	EcoRI
	Rev	GCCCAAGCTT-TTACGGGCGGTATTCGGGCT	HindIII
<b>961L</b> (pSM-214)	Fwd	CCGGAATTCATATG-AAACACTTTCCATCC	EcoRI
	Rev	GCCCAAGCTT-TTACCACTCGTAATTGAC	HindIII
<b>961</b> (pSM-214)	Fwd	CCGGAATTCATATG-GCCACAAGCGACGAC	EcoRI
	Rev	GCCCAAGCTT-TTACCACTCGTAATTGAC	HindIII
<b>961c L</b> pSM-214	Fwd	CCGGAATTCCTTATG-AAACACTTTCCATCC	EcoRI
	Rev	GCCCAAGCTT-TCAACCCACGTTGTAAGGTTG	HindIII
<b>961c</b> pSM-214	Fwd	CCGGAATTCCTTATG-GCCACAACGACGACG	EcoRI
	Rev	GCCCAAGCTT-TCAACCCACGTTGTAAGGTTG	HindIII
<b>953</b> (pSM-214)	Fwd	CCGGAATTCCTTATG-GCCACCTACAAAGTGGACGA	EcoRI
	Rev	GCCCAAGCTT-TTATTGTTTGGCTGCCTCGATT	HindIII

These sequences were manipulated, cloned and expressed as described for 953L.

For the pET-24 vector, sequences were cloned and the proteins expressed in pET-24 as described below for pET21. pET2 has the same sequence as pET-21, but with the kanamycin resistance cassette instead of ampicillin cassette.

- 5 Oligonucleotides used to clone sequences into pET-24b vector were:

<b>ΔG 287 K</b>	Fwd	<b>CGCGGATCCGCTAGC-CCCGATGTAAATCGGC</b> §	NheI
	Rev	<b>CCCGCTCGAG-TCAATCCTGCTCTTTTTTGCC</b> *	XhoI
<b>Δ2 287 K</b>	Fwd	<b>CGCGGATCCGCTAGC-CAAGATATGGCGGCAGT</b> §	NheI
<b>Δ3 287 K</b>	Fwd	<b>CGCGGATCCGCTAGC-GCCGAATCCGCAAATCA</b> §	NheI
<b>Δ4 287 K</b>	Fwd	<b>CGCGCTAGC-GGAAGGGTTGATTTGGCTAATGG</b> §	NheI
<b>Orf46.1 K</b>	Fwd	<b>GGGAATTCCATATG-GGCATTTCCCGCAAAATATC</b>	NdeI
	Rev	<b>CCCGCTCGAG-TTACGTATCATATTTACGTGC</b>	XhoI
<b>Orf46A K</b>	Fwd	<b>GGGAATTCCATATG-GGCATTTCCCGCAAAATATC</b>	NdeI
	Rev	<b>CCCGCTCGAG-TTATTCTATGCCTTGTGCGGCAT</b>	XhoI
<b>961 K (MC58)</b>	Fwd	<b>CGCGGATCCCATATG-GCCACAAGCGACGACGA</b>	NdeI
	Rev	<b>CCCGCTCGAG-TTACCACTCGTAATTGAC</b>	XhoI
<b>961a K</b>	Fwd	<b>CGCGGATCCCATATG-GCCACAACGACG</b>	NdeI
	Rev	<b>CCCGCTCGAG-TCATTTAGCAATATTATCTTTGTTC</b>	XhoI
<b>961b K</b>	Fwd	<b>CGCGGATCCCATATG-AAAGCAAACAGTGCCGAC</b>	NdeI
	Rev	<b>CCCGCTCGAG-TTACCACTCGTAATTGAC</b>	XhoI
<b>961c K</b>	Fwd	<b>CGCGGATCCCATATG-GCCACAACGACG</b>	NdeI
	Rev	<b>CCCGCTCGAG-TTAACCCACGTTGTAAGGT</b>	XhoI
<b>961cL K</b>	Fwd	<b>CGCGGATCCCATATG-ATGAAACACTTTCCATCC</b>	NdeI
	Rev	<b>CCCGCTCGAG-TTAACCCACGTTGTAAGGT</b>	XhoI
<b>961d K</b>	Fwd	<b>CGCGGATCCCATATG-GCCACAACGACG</b>	NdeI
	Rev	<b>CCCGCTCGAG-TCAGTCTGACACTGTTTATCC</b>	XhoI
<b>ΔG 287-919 K</b>	Fwd	<b>CGCGGATCCGCTAGC-CCCGATGTAAATCGGC</b>	NheI
	Rev	<b>CCCGCTCGAG-TTACGGGCGGTATTCGG</b>	XhoI
<b>ΔG 287-Orf46.1 K</b>	Fwd	<b>CGCGGATCCGCTAGC-CCCGATGTAAATCGGC</b>	NheI
	Rev	<b>CCCGCTCGAG-TTACGTATCATATTTACGTGC</b>	XhoI
<b>ΔG 287-961 K</b>	Fwd	<b>CGCGGATCCGCTAGC-CCCGATGTAAATCGGC</b>	NheI
	Rev	<b>CCCGCTCGAG-TTACCACTCGTAATTGAC</b>	XhoI

\* This primer was used as a Reverse primer for all the 287 forms.

§ Forward primers used in combination with the ΔG278 K reverse primer.

#### Example 6 – ORF1 and its leader peptide

ORF1 from *N.meningitidis* (serogroup B, strain MC58) is predicted to be an outer membrane or secreted protein. It has the following sequence:

10

1 MKTTDKRTTE THRKAPKTGR IRFSPAYLAI CLSFGILPQA WAGHTYFGIN

51 YQYYRDFAEK KGKFAVGAKD IEVYNKKGEL VGKSMKAPM IDFSVVSRRG  
 101 VAALVGDQYI VSVAHNGGYN NVDFGAEGRN PDQHRFTYKI VKRNNYKAGT  
 151 KGHYPYGGDYH MPRLHKFVTD AEPVEMTSYM DGRKYIDQNN YPDRVRIGAG  
 201 RQYWRSEDEE PNNRESSYHI ASAYSWLVG NTFAQNGSGG GTVNLGSEKI  
 5 KHSFYGFLLPT GGSFGDSGSP MFIYDAQKQK WLINGVLQGT NPYIGKSNFG  
 251 QLVKRDWFDY EIFAGDTHSV FYEPRQNGKY SFNDDNNGTG KINAKHEHNS  
 301 LPNRLKTRTV QLFNVSLSET AREPVYHAAG GVNSYRPRLN NGENISFIDE  
 351 GKGEILITSN INQAGAGLYF QGDFTVSPEN NETWQAGGVH ISEDSTVTWK  
 401 VNGVANDRLS KIGKGTLLHVQ AKGENQGSIS VGDGTVILDQ QADDKGGKQA  
 10 501 FSEIGLVSGR GTVQLNADNQ FNPDKLYFGF RGGRLDLNGH SLSFHRIQNT  
 551 DEGAMIVNHN QDKESTVTIT GNKDIATTGN NNSLDSKKEI AYNGWFGKED  
 601 TTKTNGRLNL VYQPAEDRT LLLSGGTNLN GNITQTNGKL FFSGRPTPHA  
 651 YNHLNDHWSQ KEGIPRGEIV WDNDWINRTF KAENFQIKGG QAVVSRNVAK  
 701 VKGDWHLNSH AQAVFGVAPH QSHTICTRSD WTGLTNCVEK TITDDKVIAS  
 15 751 LTKTDISGNV DLADHAHLNL TGLATLNGNL SANGDTRYTV SHNATQNGNL  
 801 SLVGNAQATF NQATLNGNTS ASGNASFNLS DHAVQNGSLT LSGNAKANVS  
 851 HSALNGNVSL ADKAVFHFES SRFTGQISGG KDTALHLKDS EWTLPSTGEL  
 901 GNLNLDNATI TLNSAYRHDA AGAQTGSATD APRRRSRRSR RSLLSVTPPT  
 951 SVESRFNTLT VNGKLNQGT FRFMSELFY RSDKLKLAES SEGTYTLAVN  
 20 1001 NTGNEPASLE QLTVEGKDN KPLSENLFNT LQNEHVDAGA WRYQLIRKDG  
 1051 EFRHLNPNVKE QELSDKLGA EAKKQAEKDN AQSLDALIAA GRDAVEKTES  
 1101 VAEPARQAGG ENVGIMQAE EKKRVQADKD TALAKQREAE TRPATTAFFR  
 1151 ARRARRDLPO LQPQPQPQPQ RDLISRYANS GLSEFSATLN SVFAVQDELD  
 25 1201 RVFAEDRRNA VWTSGIRDTH HYRSQDFRAY RQQTDLRQIG MQKNLGSGRV  
 1251 GILFSHNRTE NTDDGIGNS ARLAHGAVFG QYGIDRFYIG ISAGAGFSSG  
 1301 SLSDGIGGKI RRRVLHYGIQ ARYRAGFGGF GIEPHIGATR YFVQKADYRY  
 1351 ENVNIATPGL AFNRYRAGIK ADYSFKPAQH ISITPYLSLS YTDAASGKVR  
 1401 TRVNTAVLAQ DFGKTRSAEW GVNAEIKGFT LSLHAAAAGK PQLEAQSAG  
 1451 IKLGYRW\*

30 The leader peptide is underlined.

A polymorphic form of ORF1 is disclosed in WO99/55873.

Three expression strategies have been used for ORF1:

- 1) ORF1 using a His tag, following WO99/24578 (ORF1-His);
- 2) ORF1 with its own leader peptide but without any fusion partner ('ORF1L'); and
- 35 3) ORF1 with the leader peptide (MKKTAIAIAVALAGFATVAQA) from *E.coli* OmpA ('Orf1LOmpA'):

40 MKKTAIAIAVALAGFATVAQAASAGHTYFGINYQYYRDFAEKKGKFAVGAKDIEVYNKKGELVGKSMKAPMIDFSV  
 VSRNGVAALVGDQYIVSVAHNGGYNVNDVFGAEGRNPDQHRFTYKIVKRNNYKAGTKGHYPYGGDYHMPRLHKFVTD  
 PVENTSYMDGRKYIDQNNYVDRVRIGAGRQYWRSEDEEPNNRESSYHIASAYSWLVGNTFAQNGSGGGT  
 45 IKHSFYGFLLPTGGSFGDSGSPMFIYDAQKQKWLINGVLQGTNPYIGKSNFGQLVVRDWFYDEIFAGDTHSVFYEP  
 NGKYSFNDDNNGTGKINAKHEHNSLPNRLKTRTVQLFNVSLSETAREPVYHAAGGVNSYRPRLNNGENISFIDE  
 ELILTSNINQAGAGLYFQGDFTVSPENNETWQAGGVHISEDSTVTWKVNGVANDRLSKIGKGTLLHVQAKGENQGSIS  
 50 VGDGTVILDQADDKGGKQAFSEIGLVSGRGTVQLNADNQFNPDKLYFGFRGGRLDLNGHSLSFHRIQNTDEGAMIV  
 NHNQDKESTVTITGNKDIATTGNMNSLDSKKEIAYNGWFGKEDTTKTNGRLNLVYQPAEDRTLLLSGGTNLNGNIT  
 45 QTNGKLFFSGRPTPHAYNHLNDHWSQKEGIPRGEIVWDNDWINRTFKAENFQIKGGQAVVSRNVAKVKGDWHLNSHA  
 QAVFGVAPHQSHTICTRSDWTGLTNCVEKTITDDKVIASLTKTDISGNVDLADHAHLNLTLGLATLNGNL  
 TVSHNATQNGNLSLVGNAQATFNQATLNGNTSASGNASFNLSDHAVQNGSLTSLGNAKANVSHSALNGNVSLADKAV  
 50 FHFESSRFTGQISGGKDTALHLKDSWTLPSTGELGNLNDNATITLNSAYRHDAAGAQTGSATDAPRRRSRRSR  
 LLSVTPPTSVESRFNTLTVNGKLNQGTFRFMSELFYRSDKLKLAESSEGTYTLAVNNTGNEPASLEQLTVVEGKD  
 55 NKPLSENLFNTLQNEHVDAGAWRYQLIRKDGFRHLNPNVKEQELSDKLGAEEAKKQAEKDNQAQSLDALIAA  
 KTESVAEPARQAGGENVGIMQAEKEKKRVQADKDTALAKQREAE  
 TRPATTAFFRARRARRDLPLQPQPQPQPQORDL  
 ISRYANSGLSEFSATLNSVFAVQDELDRVFAEDRRNAVWTSGIRDTHYRSQDFRAYRQQTDLRQIGMQKNLGSGRV  
 GILFSHNRTE  
 NTDDGIGNSARLAHGAVFGQYGIDRFYIGISAGAGFSSGSLSDGIGGKIRRRVLHYGIQARYRAGF  
 55 GGFIEPHIGATRYFVQKADYRYENVNIATPGLAFNRYRAGIKADYSFKPAQHISITPYLSLSYTDASGKVRTRVN  
 TAVLAQDFGKTRSAEWGVNAEIKGFTLSLHAAAAGKQPLEAQSAGIKLGYRW\*

To make this construct, the clone pET911LOmpA (see below) was digested with the *NheI* and *XhoI* restriction enzymes and the fragment corresponding to the vector carrying the OmpA leader sequence was purified (pETLOmpA). The ORF1 gene coding for the mature protein was amplified using the oligonucleotides ORF1-For and ORF1-Rev (including the *NheI* and *XhoI* restriction sites, respectively), digested with *NheI* and *XhoI* and ligated to the purified pETOmpA fragment (see Figure 1). An additional AS dipeptide was introduced by the *NheI* site.

All three forms of the protein were expressed. The His-tagged protein could be purified and was confirmed as surface exposed, and possibly secreted (see Figure 3). The protein was used to immunise mice, and the resulting sera gave excellent results in the bactericidal assay.

ORF1LOmpA was purified as total membranes, and was localised in both the inner and outer membranes. Unexpectedly, sera raised against ORF1LOmpA show even better ELISA and anti-bactericidal properties than those raised against the His-tagged protein.

ORF1L was purified as outer membranes, where it is localised.

#### 15 **Example 7 – protein 911 and its leader peptide**

Protein 911 from *N.meningitidis* (serogroup B, strain MC58) has the following sequence:

```

1  MKKNILEFWV GLFVLIGAAA VAFLAFRVAG GAAFGGSDKT YAVYADFGDI
51  GGLKVNAPVK SAGVLVGRVG AIGLDPKSYQ ARVRLDLGK YQFSSDVSAQ
101 ILTSGLLGEQ YIGLQQGGDT ENLAAGDTIS VTSSAMVLEN LIGKFMTSFA
151 EKNADGGNAE KAAE*
```

The leader peptide is underlined.

Three expression strategies have been used for 911:

- 1) 911 with its own leader peptide but without any fusion partner ('911L');
- 2) 911 with the leader peptide from *E.coli* OmpA ('911LOmpA').

To make this construct, the entire sequence encoding the OmpA leader peptide was included in the 5'- primer as a tail (primer 911LOmpA Forward). A *NheI* restriction site was inserted between the sequence coding for the OmpA leader peptide and the 911 gene encoding the predicted mature protein (insertion of one amino acid, a serine), to allow the use of this construct to clone different genes downstream the OmpA leader peptide sequence.

- 3) 911 with the leader peptide (MKYLLPTAAAGLLLAQPAMA) from *Erwinia carotovora* PelB ('911LpelB').

To make this construct, the 5'-end PCR primer was designed downstream from the leader sequence and included the *NcoI* restriction site in order to have the 911 fused directly to the PelB leader sequence; the 3'- end primer included the STOP codon. The expression vector used was pET22b+ (Novagen), which carries the coding sequence for the PelB leader peptide. The *NcoI* site introduces an additional methionine after the PelB sequence.

All three forms of the protein were expressed. ELISA titres were highest using 911L, with 919LOmpA also giving good results.

### Example 8 – ORF46

The complete ORF46 protein from *N.meningitidis* (serogroup B, strain 2996) has the following sequence:

```

1  LGISRKISLI LSILAVCLPM HAHASDLAND SFIRQVLDRO HFEPDGKYHL
51  FGSRGELAER SGHIGLGKIQ SHQLGNLMIQ QAAIKGNIGY IVRFS DHGHE
101 VHSPPDNHAS HSDSDEAGSP VDGFSLYRIH WDGYEHPAD GYDGPQGGGY
151 PAPKGARDIY SYDIKVAQN IRLNLTNRS TGQRLADRFH NAGSMLTQGV
201 GDGFKRATRY SPELDRSGNA AEA FNGTADI VKNIIGAAGE IVGAGDAVQG
251 ISEGSNIAVM HGLGLLSTEN KMARINDLAD MAQLKDYAAA AIRDWAVQNP
301 NAAQGIEAVS NIFMAAIPK GIGAVRGKYG LGGITAHPIK RSQMGAIALP
351 KGKSAVSDNF ADAAYAKYPS PYHSRNIRSN LEQRYGKENI TSSTVPPSNG
20 401 KNVKLADQRH PKTGVPFDGK GFPNFEKHVK YDTKLDIQEL SGGGIPKAKP
451 VSDAKPRWEV DRKLNKLTR EQVEKNVQEI RGNKNNSNFS QHAQLEREIN
501 KLKSADEINF ADGMGKFTDS MNDKAFSRLV KSVKENGFTN PVVEYVEING
551 KAYIVRGNNR VFAAEYLGR I HELKFKKVDF PVPNTSWKNP TDVLN ESGNV
25 601 KRPRYRSK*

```

The leader peptide is underlined.

The sequences of ORF46 from other strains can be found in WO00/66741.

Three expression strategies have been used for ORF46:

- 1) ORF46 with its own leader peptide but without any fusion partner ('ORF46-2L');
- 2) ORF46 without its leader peptide and without any fusion partner ('ORF46-2'), with the leader peptide omitted by designing the 5'-end amplification primer downstream from the predicted leader sequence:

```

35 1  SDLANDSFIR QVLDROHFEP DGKYHLFGSR GELAERSGHI GLGKIQSHQL
51  GNLMIQQAAT KGNIGYIVRF SDHGHEVHSP FDNHASHSDS DEAGSPVDGF
101 SLYRIHWDGY EHHPADGYDG PQGGGYPAKP GARDIYSYDI KGVAQNIRLN
151 LTDNRSTGQR LADRFHNAGS MLTQGVGDGF KRATRYSPEL DRSGNAAEAF
201 NGTADIVKNI IGAAGEIVGA GDAVQGISEG SNIAVMHGLG LLSTENKMAR
251 INDLADMAQL KDYAAAAIRD WAVQNPNAAG GIEAVSNIFM AAIPKIGIGA
301 VRGKYGLGGI TAHPIKRSQM GAIALPKGKS AVSDNFADAA YAKYPSPYHS
40 351 RNIRSNLEQR YGKENITSST VPPSNGKNVK LADQRHPKTG VPFDGKGFPN
401 FEKHVKYDTK LDIQELSGGG IPKAKPVSDA KPRWEVDRKL NKLTTREQVE
451 KNVQEI RGN KNSNFSQHAQ LEREINKLKS ADEINFADGM GKFTDSMNDK
501 AFSRLVKSVK ENGFTNPVVE YVEINGKAYI VRGNRNVFAA EYLGRIHELK
551 FKKVDFPVPN TSWKNPTDVL NESGNVKRPR YRSK*

```

- 3) ORF46 as a truncated protein, consisting of the first 433 amino acids ('ORF46.1L'), constructed by designing PCR primers to amplify a partial sequence corresponding to aa 1-433.

5 A STOP codon was included in the 3'-end primer sequences.

ORF46-2L is expressed at a very low level to *E.coli*. Removal of its leader peptide (ORF46-2) does not solve this problem. The truncated ORF46.1L form (first 433 amino acids, which are well conserved between serogroups and species), however, is well-expressed and gives excellent results in ELISA test and in the bactericidal assay.

- 10 ORF46.1 has also been used as the basis of hybrid proteins. It has been fused with 287, 919, and ORF1. The hybrid proteins were generally insoluble, but gave some good ELISA and bactericidal results (against the homologous 2996 strain):

Protein	ELISA	Bactericidal Ab
Orf1-Orf46.1-His	850	256
919-Orf46.1-His	12900	512
919-287-Orf46-His	n.d.	n.d.
Orf46.1-287His	150	8192
Orf46.1-919His	2800	2048
Orf46.1-287-919His	3200	16384

- For comparison, 'triple' hybrids of ORF46.1, 287 (either as a GST fusion, or in  $\Delta$ G287 form) and 919 were constructed and tested against various strains (including the homologous 2996 strain) *versus* a simple mixture of the three antigens. FCA was used as adjuvant:

	2996	BZ232	MC58	NGH38	F6124	BZ133
Mixture	8192	256	512	1024	>2048	>2048
ORF46.1-287-919his	16384	256	4096	8192	8192	8192
$\Delta$ G287-919-ORF46.1his	8192	64	4096	8192	8192	16384
$\Delta$ G287-ORF46.1-919his	4096	128	256	8192	512	1024

Again, the hybrids show equivalent or superior immunological activity.

Hybrids of two proteins (strain 2996) were compared to the individual proteins against various heterologous strains:

	1000	MC58	F6124 (MenA)
ORF46.1-His	<4	4096	<4
ORF1-His	8	256	128
ORF1—ORF46.1-His	1024	512	1024

Again, the hybrid shows equivalent or superior immunological activity.

### Example 9 – protein 961

The complete 961 protein from *N.meningitidis* (serogroup B, strain MC58) has the following sequence:

```

5      1  MSMKHFPAKV LTTAILATFC SGALAATSDD DVKKAATVAI VAAYNNGQEI
      51  NGFKAGETIY DIGEDGTITQ KDATAADVEA DDFKGLGLKK VVTNLTKTVN
     101  ENKQNVDAKV KAAESEIEKL TTKLADTDAA LADTDAALDE TTNALNKLGE
     151  NITTFAEETK TNIVKIDEKL EAVADTVDKH AEA FN DIADS LDETNTKADE
     201  AVKTANEARQ TAEETKQNV D AKV KAAETAA GKAEAAAGTA NTAADKAEAV
     251  AAKVTDIKAD IATNKADIAK NSARIDSLDK NVANLRKETR QGLAEQAALS
     301  GLFQPYNVGR FNVTAAVGGY KSESAVAIGT GFRFTENFAA KAGVAVGTSS
     351  GSSAAYHGV NYEW*
```

The leader peptide is underlined.

15 Three approaches to 961 expression were used:

- 1) 961 using a GST fusion, following WO99/57280 ('GST961');
- 2) 961 with its own leader peptide but without any fusion partner ('961L'); and
- 3) 961 without its leader peptide and without any fusion partner ('961<sup>untagged</sup>'), with the leader peptide omitted by designing the 5'-end PCR primer downstream from the

20 predicted leader sequence.

All three forms of the protein were expressed. The GST-fusion protein could be purified and antibodies against it confirmed that 961 is surface exposed (Figure 4). The protein was used to immunise mice, and the resulting sera gave excellent results in the bactericidal assay. 961L could also be purified and gave very high ELISA titres.

25 Protein 961 appears to be phase variable. Furthermore, it is not found in all strains of *N.meningitidis*.

### Example 10 – protein 287

Protein 287 from *N.meningitidis* (serogroup B, strain 2996) has the following sequence:

```

30      1  MFERSVIAMA CIFALSACGG GGGGSPDVKS ADTLSKPAAP VVAEKETEVEK
      51  EDAPQAGSQG QGAPSTQGSQ DMAAVSAENT GNGGAATTDK PKNEDEGPQN
     101  DMPQNSAESA NQTGNNQPAD SSDSAPASNP APANGGSNFG RVDLANGVLI
     151  DGPSQNITLT HCKGDSCNGD NLLDEEAPSK SEFENLNESE RIEKYKKGDK
```

5                   201    SDKFTNLVAT AVQANGTNKY VLIYKDKSAS SSSARFRSA RSRRSLPAEM  
                   251    PLIPVNQADT LIVDGEAVSL TGHSGNIFAP EGNRYRLTYG AEKLPGGSYA  
                   301    LRVQGEPAKG EMLAGTAVYN GEVLHFHTEN GRPYPTRGRF AAKVDFGSKS  
                   351    VDGIIDSGDD LHMGTQKFKA AIDGNGFKGT WTENGGGDVS GRFYGPAGEE  
                   401    VAGKYSYRPT DAERGGFGVF AGKKEQD\*

The leader peptide is shown underlined.

The sequences of 287 from other strains can be found in Figures 5 and 15 of WO00/66741.

Example 9 of WO99/57280 discloses the expression of 287 as a GST-fusion in *E.coli*.

10   A number of further approaches to expressing 287 in *E.coli* have been used, including:

- 1) 287 as a His-tagged fusion ('287-His');
- 2) 287 with its own leader peptide but without any fusion partner ('287L');
- 3) 287 with the ORF4 leader peptide and without any fusion partner ('287Lorf4'); and
- 4) 287 without its leader peptide and without any fusion partner ('287<sup>untagged</sup>');

15                   1    CGGGGGGSPD VKSADTLSP AAPVVAEKET EVKEDAPQAG SQGQGAPSTQ  
                   51    GSQDMAAVSA ENTGNGGAAT TDKPKNEDEG PQNDMPQNSA ESANQTGNNQ  
                   101   PADSSDSAPA SNPAPANGGS NFGRVDLANG VLIDGPSQNI TLTHCKGDSC  
                   151   NGDNLLDEEA PSKSEFENLN ESERIEKYKK DGKSDKFTNL VATAVQANGT  
                   201   NKYVLIYKDK SASSSSARFR RSARSRRSLP AEMPLIPVNQ ADTLIVDGEA  
 20                   251   VSLTGHSGNI FAPEGNYRYL TYGAEKLPQG SYALRVQGEP AKGEMLAGTA  
                   301   VYNGEVLHFH TENGRPYPTR GRFAAKVDFG SKSVDGIIDS GDDLHMGTOK  
                   351   FKAIDGNGF KGTWTENGGG DVSGRFYGPA GEEVAGKYSY RPTDAEKGGF  
                   401   GVFAGKKEQD \*

25   All these proteins could be expressed and purified.

'287L' and '287Lorf4' were confirmed as lipoproteins.

As shown in Figure 2, '287Lorf4' was constructed by digesting 919Lorf4 with *NheI* and *XhoI*. The entire ORF4 leader peptide was restored by the addition of a DNA sequence coding for the missing amino acids, as a tail, in the 5'-end primer (287Lorf4 for), fused to  
 30   287 coding sequence. The 287 gene coding for the mature protein was amplified using the oligonucleotides 287Lorf4 For and Rev (including the *NheI* and *XhoI* sites, respectively), digested with *NheI* and *XhoI* and ligated to the purified pETOrf4 fragment.

**Example 11 – further non-fusion proteins with/without native leader peptides**

A similar approach was adopted for *E.coli* expression of further proteins from WO99/24578,  
 35   WO99/36544 and WO99/57280.

The following were expressed without a fusion partner: 008, 105, 117-1, 121-1, 122-1, 128-1, 148, 216, 243, 308, 593, 652, 726, 982, and Orf143-1. Protein 117-1 was confirmed as surface-exposed by FACS and gave high ELISA titres.

The following were expressed with the native leader peptide but without a fusion partner:  
5 111, 149, 206, 225-1, 235, 247-1, 274, 283, 286, 292, 401, 406, 502-1, 503, 519-1, 525-1, 552, 556, 557, 570, 576-1, 580, 583, 664, 759, 907, 913, 920-1, 926, 936-1, 953, 961, 983, 989, Orf4, Orf7-1, Orf9-1, Orf23, Orf25, Orf37, Orf38, Orf40, Orf40.1, Orf40.2, Orf72-1, Orf76-1, Orf85-2, Orf91, Orf97-1, Orf119, Orf143.1. These proteins are given the suffix 'L'.

His-tagged protein 760 was expressed with and without its leader peptide. The deletion of  
10 the signal peptide greatly increased expression levels. The protein could be purified most easily using 2M urea for solubilisation.

His-tagged protein 264 was well-expressed using its own signal peptide, and the 30kDa protein gave positive Western blot results.

All proteins were successfully expressed.

15 The localisation of 593, 121-1, 128-1, 593, 726, and 982 in the cytoplasm was confirmed.

The localisation of 920-1L, 953L, ORF9-1L, ORF85-2L, ORF97-1L, 570L, 580L and 664L in the periplasm was confirmed.

The localisation of ORF40L in the outer membrane, and 008 and 519-1L in the inner membrane was confirmed. ORF25L, ORF4L, 406L, 576-1L were all confirmed as being  
20 localised in the membrane.

Protein 206 was found not to be a lipoprotein.

ORF25 and ORF40 expressed with their native leader peptides but without fusion partners, and protein 593 expressed without its native leader peptide and without a fusion partner, raised good anti-bactericidal sera. Surprisingly, the forms of ORF25 and ORF40 expressed  
25 without fusion partners and using their own leader peptides (*i.e.* 'ORF25L' and 'ORF40L') give better results in the bactericidal assay than the fusion proteins.

Proteins 920L and 953L were subjected to N-terminal sequencing, giving HRVWVETAH and ATYKVDEYHANARFAF, respectively. This sequencing confirms that the predicted leader peptides were cleaved and, when combined with the periplasmic location, confirms that the

proteins are correctly processed and localised by *E.coli* when expressed from their native leader peptides.

The N-terminal sequence of protein 519.1L localised in the inner membrane was MEFFIILLA, indicating that the leader sequence is not cleaved. It may therefore function as both an  
5   uncleaved leader sequence and a transmembrane anchor in a manner similar to the leader peptide of PBP1 from *N.gonorrhoeae* [Ropp & Nicholas (1997) *J. Bact.* 179:2783-2787.]. Indeed the N-terminal region exhibits strong hydrophobic character and is predicted by the Tmpred. program to be transmembrane.

### **Example 12 – lipoproteins**

10   The incorporation of palmitate in recombinant lipoproteins was demonstrated by the method of Kraft *et. al.* [*J. Bact.* (1998) 180:3441-3447.]. Single colonies harbouring the plasmid of interest were grown overnight at 37°C in 20 ml of LB/Amp (100µg/ml) liquid culture. The culture was diluted to an OD<sub>550</sub> of 0.1 in 5.0 ml of fresh medium LB/Amp medium containing 5 µC/ml [<sup>3</sup>H] palmitate (Amersham). When the OD<sub>550</sub> of the culture reached 0.4-  
15   0.8, recombinant lipoprotein was induced for 1 hour with IPTG (final concentration 1.0 mM). Bacteria were harvested by centrifugation in a bench top centrifuge at 2700g for 15 min and washed twice with 1.0 ml cold PBS. Cells were resuspended in 120µl of 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1.0% w/v SDS and lysed by boiling for 10 min. After centrifugation at 13000g for 10 min the supernatant was collected and proteins precipitated  
20   by the addition of 1.2 ml cold acetone and left for 1 hour at -20 °C. Protein was pelleted by centrifugation at 13000g for 10 min and resuspended in 20-50µl (calculated to standardise loading with respect to the final O.D of the culture) of 1.0% w/v SDS. An aliquot of 15 µl was boiled with 5µl of SDS-PAGE sample buffer and analysed by SDS-PAGE. After electrophoresis gels were fixed for 1 hour in 10% v/v acetic acid and soaked for 30 minutes  
25   in Amplify solution (Amersham). The gel was vacuum-dried under heat and exposed to Hyperfilm (Kodak) overnight -80 °C.

Incorporation of the [<sup>3</sup>H] palmitate label, confirming lipidation, was found for the following proteins: Orf4L, Orf25L, 287L, 287LOrf4, 406.L, 576L, 926L, 919L and 919LOrf4.

### **Example 13 – domains in 287**

30   Based on homology of different regions of 287 to proteins that belong to different functional classes, it was split into three 'domains', as shown in Figure 5. The second domain shows

homology to IgA proteases, and the third domain shows homology to transferrin-binding proteins.

Each of the three 'domains' shows a different degree of sequence conservation between *N.meningitidis* strains – domain C is 98% identical, domain A is 83% identical, whilst domain B is only 71% identical. Note that protein 287 in strain MC58 is 61 amino acids longer than that of strain 2996. An alignment of the two sequences is shown in Figure 7, and alignments for various strains are disclosed in WO00/66741 (see Figures 5 and 15 therein).

The three domains were expressed individually as C-terminal His-tagged proteins. This was done for the MC58 and 2996 strains, using the following constructs:

- 10           287a-MC58 (aa 1-202), 287b-MC58 (aa 203-288), 287c-MC58 (aa 311-488).  
               287a-2996 (aa 1-139), 287b-2996 (aa 140-225), 287c-2996 (aa 250-427).

To make these constructs, the stop codon sequence was omitted in the 3'-end primer sequence. The 5' primers included the *NheI* restriction site, and the 3' primers included a *XhoI* as a tail, in order to direct the cloning of each amplified fragment into the expression vector pET21b+ using *NdeI-XhoI*, *NheI-XhoI* or *NdeI-HindIII* restriction sites.

All six constructs could be expressed, but 287b-MC8 required denaturation and refolding for solubilisation.

Deletion of domain A is described below ('Δ4 287-His').

Immunological data (serum bactericidal assay) were also obtained using the various domains from strain 2996, against the homologous and heterologous MenB strains, as well as MenA (F6124 strain) and MenC (BZ133 strain):

	2996	BZ232	MC58	NGH38	394/98	MenA	MenC
<b>287-His</b>	32000	16	4096	4096	512	8000	16000
<b>287(B)-His</b>	256	-	-	-	-	16	-
<b>287(C)-His</b>	256	-	32	512	32	2048	>2048
<b>287(B-C)-His</b>	64000	128	4096	64000	1024	64000	32000

Using the domains of strain MC58, the following results were obtained: .

	MC58	2996	BZ232	NGH38	394/98	MenA	MenC
<b>287-His</b>	4096	32000	16	4096	512	8000	16000
<b>287(B)-His</b>	128	128	-	-	-	-	128
<b>287(C)-His</b>	-	16	-	1024	-	512	-
<b>287(B-C)-His</b>	16000	64000	128	64000	512	64000	>8000

**Example 14 – deletions in 287**

As well as expressing individual domains, 287 was also expressed (as a C-terminal His-tagged protein) by making progressive deletions within the first domain. These

Four deletion mutants of protein 287 from strain 2996 were used (Figure 6):

- 5           1) '287-His', consisting of amino acids 18-427 (*i.e.* leader peptide deleted);
- 2) 'Δ1 287-His', consisting of amino acids 26-427;
- 3) 'Δ2 287-His', consisting of amino acids 70-427;
- 4) 'Δ3 287-His', consisting of amino acids 107-427; and
- 5) 'Δ4 287-His', consisting of amino acids 140-427 (=287-bc).
- 10       The 'Δ4' protein was also made for strain MC58 ('Δ4 287MC58-His'; aa 203-488).

The constructs were made in the same way as 287a/b/c, as described above.

All six constructs could be expressed and protein could be purified. Expression of 287-His was, however, quite poor.

Expression was also high when the C-terminal His-tags were omitted.

- 15       Immunological data (serum bactericidal assay) were also obtained using the deletion mutants, against the homologous (2996) and heterologous MenB strains, as well as MenA (F6124 strain) and MenC (BZ133 strain):

	2996	BZ232	MC58	NGH38	394/98	MenA	MenC
<b>287-his</b>	32000	16	4096	4096	512	8000	16000
<b>Δ1 287-His</b>	16000	128	4096	4096	1024	8000	16000
<b>Δ2 287-His</b>	16000	128	4096	>2048	512	16000	>8000
<b>Δ3 287-His</b>	16000	128	4096	>2048	512	16000	>8000
<b>Δ4 287-His</b>	64000	128	4096	64000	1024	64000	32000

The same high activity for the Δ4 deletion was seen using the sequence from strain MC58.

As well as showing superior expression characteristics, therefore, the mutants are immunologically equivalent or superior.

### Example 15 – poly-glycine deletions

The 'Δ1 287-His' construct of the previous example differs from 287-His and from '287<sup>untagged</sup>', only by a short N-terminal deletion (GGGGGGS). Using an expression vector which replaces the deleted serine with a codon present in the *Nhe* cloning site, however, this amounts to a deletion only of (Gly)<sub>6</sub>. Thus, the deletion of this (Gly)<sub>6</sub> sequence has been shown to have a dramatic effect on protein expression.

The protein lacking the N-terminal amino acids up to GGGGGG is called 'ΔG 287'. In strain MC58, its sequence (leader peptide underlined) is:

⇨ ΔG287

	1	<u>MFKRSVIAMA</u>	<u>CIFALSACGG</u>	<u>GGGGSPDVKS</u>	<u>ADTLSPAAP</u>	<u>VVSEKETEAK</u>
	51	<u>EDAPQAGSQ</u>	<u>QGAPSAQGSQ</u>	<u>DMAAVSEENT</u>	<u>GNGGAVTADN</u>	<u>PKNEDEVAQN</u>
15	101	<u>DMPQNAAGTD</u>	<u>SSTPNHTPDP</u>	<u>NMLAGNMENQ</u>	<u>ATDAGESSQP</u>	<u>ANQPDMANAA</u>
	151	<u>DGMQGGDDPSA</u>	<u>GGQNAGNTAA</u>	<u>QGANQAGNNQ</u>	<u>AAGSSDPIPA</u>	<u>SNPAPANGGS</u>
	201	<u>NFGRVDLANG</u>	<u>VLIDGPSQNI</u>	<u>TLTHCKGDS</u>	<u>SGNNFLDEEV</u>	<u>QLKSEFEKLS</u>
	251	<u>DADKISNYKK</u>	<u>DGKNDKFVGL</u>	<u>VADSVQMKGI</u>	<u>NOYIIFYKPK</u>	<u>PTSFARFRRS</u>
	301	<u>ARSRRSLPAE</u>	<u>MPLIPVNQAD</u>	<u>TLIVDGEAVS</u>	<u>LTGHSGNIFA</u>	<u>PEGNYRYLTY</u>
	351	<u>GAEKLPGGSY</u>	<u>ALRVQGEPAK</u>	<u>GEMLAGAAVY</u>	<u>NGEVLHFHTE</u>	<u>NGRPYPTRGR</u>
20	401	<u>FAAKVDFGSK</u>	<u>SVDGIIDSGD</u>	<u>DLHMGTOQFK</u>	<u>AAIDGNGFKG</u>	<u>TWTENGSGDV</u>
	451	<u>SGKFYGPAGE</u>	<u>EVAGKYSYRP</u>	<u>TDAEKGGFGV</u>	<u>FAGKKEQD*</u>	

ΔG287, with or without His-tag ('ΔG287-His' and 'ΔG287K', respectively), are expressed at very good levels in comparison with the '287-His' or '287<sup>untagged</sup>'.

On the basis of gene variability data, variants of ΔG287-His were expressed in *E.coli* from a number of MenB strains, in particular from strains 2996, MC58, 1000, and BZ232. The results were also good.

It was hypothesised that poly-Gly deletion might be a general strategy to improve expression. Other MenB lipoproteins containing similar (Gly)<sub>n</sub> motifs (near the N-terminus, downstream of a cysteine) were therefore identified, namely Tbp2 (NMB0460), 741 (NMB1870) and 983 (NMB1969):

⇨ ΔGTbp2

		<b>TBP2</b>				
	1	<u>MNNPLVNQAA</u>	<u>MVLVPVFLLSA</u>	<u>CLGGGGSFDL</u>	<u>DSVDTEAPRP</u>	<u>APKYQDVFS</u>
	51	<u>KPQAQKDQGG</u>	<u>YGFAMRLKRR</u>	<u>NWYPQAKED</u>	<u>VKLDESDEWA</u>	<u>TGLPDEPKEL</u>
35	101	<u>PKRQKSVIEK</u>	<u>VETDSDNNIY</u>	<u>SSPYLKPSNH</u>	<u>QNGNTGNGIN</u>	<u>QPKNQAKDYE</u>
	151	<u>NFKYVYSGWF</u>	<u>YKHAKREFNL</u>	<u>KVEPKSAKNG</u>	<u>DDGYIFYHKG</u>	<u>EPSRQLPASG</u>
	201	<u>KITYKGVWHF</u>	<u>ATDTKKGQKF</u>	<u>REIIQPSKSQ</u>	<u>GDRYSGFSGD</u>	<u>DGEEYSNNK</u>
	251	<u>STLTDGQEGY</u>	<u>GFTSNLEVD</u>	<u>HNKLTGKLI</u>	<u>RNNANTDNNQ</u>	<u>ATTTOYYSLE</u>
	301	<u>AQVTGNRFNG</u>	<u>KATATDKPQQ</u>	<u>NSETKEHPFV</u>	<u>SDSSSLSGGF</u>	<u>FGPQGEELGF</u>
40	351	<u>RFLSDDQKVA</u>	<u>VVGSAKTKDK</u>	<u>PANGNTAAAS</u>	<u>GGTDAASNG</u>	<u>AAGTSSENGK</u>
	401	<u>LTTVLDAVEL</u>	<u>KLGDKEVQKL</u>	<u>DNFSNAAQLV</u>	<u>VDGIMIPLLP</u>	<u>EASESGNNQA</u>
	451	<u>NQGTNGGTAF</u>	<u>TRKFDHTPES</u>	<u>DKKDAQAGTQ</u>	<u>TNGAQTASNT</u>	<u>AGDTNGKTKT</u>

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501 YEVEVCCSNL NYLKYGMLTR KNSKSAMQAG ESSSQADART EQVEQSMFLQ  
 551 GERTDEKEIP SEQNIVYRGS WYGYIANDKS TSWSGNASNA TSGNRAEFTV  
 601 NFADKKITGT LTADNRQEAT FTIDGNIKDN GFEGTAKTAE SGFDLDQSNT  
 651 TRTPKAYITD AKVQGGFYGP KAEBLGGWFA YPGDKQTKNA TNASGNSSAT  
 701 VVFGAKRQQP VR\*

741

ΔG741

1 VNRTAFCCLS LTTALILTAC SSGGGGVAAD IGAGLADALT APLDHKDKGL  
 51 QSLTLDQSVR KNEKLKLAAG GAEKTYGNGD SLNTGKLNKD KVSRFDFIRQ  
 101 IEVDGQLITL ESGEFQVYKQ SHSALTAFQT EQIQDSEHSG KMAKROFRI  
 151 GDIAGEHTSF DKLPEGGRAT YRGTAFGSDD AGGKLTYTID FAAKQGNKGI  
 201 EHLKSPELNV DLAAADIKPD GKRHAIVSGS VLYNQAEKGS YSLGIFGGKA  
 251 QEVAGSAEVK TVNGIRHIGL AAKQ\*

15

983

ΔG983

1 MRRTPTFPTK TFKPTAMALA VATTLSACLG GGGGGTSAPD FNAGGTGIGS  
 51 NSRATTAKSA AVSYAGIKNE MCKDRSMLCA GRDDVAVTDR DAKINAPPPN  
 101 LHTGDFPNPN DAYKNLINLK PAIEAGYTGR GVEVGIVDTG ESVGSISFPE  
 151 LYGRKEHGYN ENYKNYTAYM RKEAPEDGGG KDIEASFDD EAVIETAKPT  
 201 DIRHVKEIGH IDLVSHIIGG RSVDRPAGG IAPDATLHIM NTNDETKNEM  
 251 MVAAIRNAWV KLGERGVRIV NNSFGTTSRA GTADLFQIAN SEEQYRQALL  
 301 DYSGGDKTDE GIRLMQSDY GNLSYHIRNK NMLFIFSTGN DAQAQPNNTYA  
 351 LLPFYEKDAQ KGIITVAGVD RSCEKFKREM YGEPGTEPLE YGSNHCGITA  
 401 MWCLSAPYEA SVRFTRTNPI QIAGTSFSAP IVTGTAALLL QKYPWMSNDN  
 451 LRTTLLTTAQ DIGAVGVDSK FGWGLLDACK AMNGPASFPF GDPTADTKGT  
 501 SDIAYSFRND ISGTGGLIKK GGSQQLQHG NNTYTGRITIE GGSVLVLYGNN  
 551 KSDMRVETKG ALIYNGAASG GSLNSDGIVY LADTDQSGAN ETVHIKGSLO  
 601 LDGKGTLYTR LGKLLKVDGT AIIGGKLYMS ARGKGAGYLN STGRRVPFELS  
 651 AAKIGQDYSF FTNIETDGG LSLDSVEKT AGSEGDLSY YVRRGNAART  
 701 ASAAAHSAAPA GLKHAVERQG SNLENLMVEL DASESSATPE TVETAAADRT  
 751 DMPGIRPYGA TFRAAAAVQH ANAADGVRIE NSLAATVYAD STAAHADMQG  
 801 RRLKAVSDGL DHNGTGLRVI AQTQDGGTW EQGGVEGKMR GSTQTVGIAA  
 851 KTGENTTAAA TLGMGRSTWS ENSANAKTDS ISLFAGIRHD AGDIGYLGKL  
 901 FSYGRYKNSI SRSTGADEHA EGSVNGTLMQ LGALGGVNVP FAATGDLTVE  
 951 GGLRYDLLKQ DAFKAGSAL GWSGNSLTEG TLVGLAGLKL SQPLSDKAVL  
 1001 FATAGVERDL NGRDYTVTGG FTGATAATGK TGARNMPHTR LVAGLGADVE  
 1051 FGNGWNGLAR YSYAGSKQYG NHSGRVGVGY RF\*

Tbp2 and 741 genes were from strain MC58; 983 and 287 genes were from strain 2996.  
 40 These were cloned in pET vector and expressed in *E. coli* without the sequence coding for their leader peptides or as "ΔG forms", both fused to a C-terminal His-tag. In each case, the same effect was seen – expression was good in the clones carrying the deletion of the poly-glycine stretch, and poor or absent if the glycines were present in the expressed protein:

ORF	Express.	Purification	Bact. Activity
287-His(2996)	+/-	+	+
'287 <sup>untagged</sup> '(2996)	+/-	nd	nd
$\Delta$ G287-His(2996)	+	+	+
$\Delta$ G287K(2996)	+	+	+
$\Delta$ G287-His(MC58)	+	+	+
$\Delta$ G287-His(1000)	+	+	+
$\Delta$ G287-His(BZ232)	+	+	+
Tbp2-His(MC58)	+/-	nd	nd
$\Delta$ GTbp2-His(MC58)	+	+	
741-His(MC58)	+/-	nd	nd
$\Delta$ G741-His(MC58)	+	+	
983-His (2996)			
$\Delta$ G983-His (2996)	+	+	

SDS-PAGE of the proteins is shown in Figure 13.

#### *$\Delta$ G287 and hybrids*

$\Delta$ G287 proteins were made and purified for strains MC58, 1000 and BZ232. Each of these gave high ELISA titres and also serum bactericidal titres of >8192.  $\Delta$ G287K, expressed from pET-24b, gave excellent titres in ELISA and the serum bactericidal assay.  $\Delta$ G287-ORF46.1K may also be expressed in pET-24b.

$\Delta$ G287 was also fused directly in-frame upstream of 919, 953, 961 (sequences shown below) and ORF46.1:

<u><math>\Delta</math>G287-919</u>	
10	1 ATGGCTAGCC CCGATGTTAA ATCGGCGGAC ACGCTGTCAA AACCGGCCGC
	51 TCCTGTTGTT GCTGAAAAAG AGACAGAGGT AAAAGAAGAT GCGCCACAGG
	101 CAGGTTCTCA AGGACAGGGC GCGCCATCCA CACAAGGCAG CCAAGATATG
	151 GCGGCAGTTT CGGCAGAAAA TACAGGCAAT GGCGGTGCGG CAACAACGGA
	201 CAAACCCAAA AATGAAGACG AGGGACCGCA AAATGATATG CCGCAAAATT
15	251 CCGCCGAATC CGCAAATCAA ACAGGGAACA ACCAACCCGC CGATTCTTCA
	301 GATTCCGCCC CCGCGTCAAA CCCTGCACCT GCGAATGGCG GTAGCAATTT
	351 TGGAAGGGTT GATTTGGCTA ATGGCGTTTT GATTGATGGG CCGTCGCAAA
	401 ATATAACGTT GACCCACTGT AAAGGCGATT CTTGTAATGG TGATAATTTA
	451 TTGGATGAAG AAGCACCGTC AAAATCAGAA TTTGAAAATT TAAATGAGTC
20	501 TGAACGAATT GAGAAATATA AGAAAGATGG GAAAAGCGAT AAATTTACTA
	551 ATTTGGTTCG GACAGCAGTT CAAGCTAATG GAACTAACAA ATATGTCATC
	601 ATTTATAAAG ACAAGTCCGC TTCATCTTCA TCTGCGCGAT TCAGGCGTTC
	651 TGCACGGTCG AGGAGGTCGC TTCTTGCCGA GATGCCGCTA ATCCCCGTCA
	701 ATCAGGCGGA TACGCTGATT GTCGATGGGG AAGCGGTCAG CCTGACGGGG
25	751 CATTCGGGCA ATATCTTCGC GCCCGAAGGG AATTACCGGT ATCTGACTTA
	801 CCGGGCGGAA AAATTGCCCG GCGGATCGTA TGCCCTCCGT GTGCAAGGCG
	851 AACCGGCAAA AGGCGAAATG CTTGCTGGCA CGGCCGTGTA CAACGGCGAA
	901 GTGCTGCATT TTCATACGGA AAACGGCCGT CCGTACCGA CTAGAGGCGA
	951 GTTTGCCGCA AAAGTCGATT TCGGCAGCAA ATCTGTGGAC GGCATTATCG
30	1001 ACAGCGGCGA TGATTTGCAT ATGGGTACGC AAAAATTCAA AGCCGCCATC

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1051 GATGGAAACG GCTTTAAGGG GACTTGGACG GAAAATGGCG GCGGGGATGT  
 1101 TTCCGGAAGG TTTTACGGCC CGGCCGGCGA GGAAGTGGCG GGAAAATACA  
 1151 GCTATCGCCC GACAGATGCG GAAAAGGGCG GATTTCGGCGT GTTTGCCGGC  
 1201 AAAAAAGAGC AGGATGGATC CGGAGGAGGA GGATGCCAAA GCAAGAGCAT  
 1251 CCAAACCTTT CCGCAACCCG ACACATCCGT CATCAACGGC CCGGACCGGC  
 1301 CGGTTCGGCAT CCCCAGCCCC GCCGGAACGA CGGTTCGGCGG CCGCGGGGCC  
 1351 GTCTATACCG TTGTACCGCA CCTGTCCCTG CCCCCTGGG CCGCGCAGGA  
 1401 TTTTCGCCAA AGCCTGCAAT CCTTCCGCCT CGGCTGCGCC AATTTGAAAA  
 1451 ACCGCCAAGG CTGGCAGGAT GTGTGCGCCC AAGCCTTTCA AACCCCGGTC  
 1501 CATTCCCTTC AGGCAAAACA GTTTTGTGAA CGCTATTTCA CGCCGTGGCA  
 1551 GGTTCAGGC AACGGAAGCC TTGCCGGTAC GGTACCAGG TATTACGAGC  
 1601 CGGTGCTGAA GGGCGACGAC AGGCGGACGG CACAAGCCCG CTTCCCGATT  
 1651 TACGGTATTC CCGACGATTT TATCTCCGTC CCCCTGCCTG CCGGTTTTCG  
 1701 GAGCGGAAAA GCCCTTGTCC GCATCAGGCA GACGGGAAAA AACAGCGGCA  
 1751 CAATCGACAA TACCGCGCGC ACACATACCG CCGACCTCTC CCGATTCCCC  
 1801 ATCACCAGCG GCACAACGGC AATCAAAGGC AGGTTTGAAG GAAGCCGCTT  
 1851 CCTCCCTTAC CACACGCGCA ACCAAATCAA CGGCGGCGCG CTTGACGGCA  
 1901 AAGCCCCGAT ACTCGGTAC GCCGAAGACC CCGTCGAAC TTTTATG  
 1951 CACATCCAAG GCTCGGGCCG TCTGAAAACC CCGTCCGGCA AATACATCCG  
 2001 CATCGGCTAT GCCGACAAAA ACGAACATCC CTACGTTTCC ATCGGACGCT  
 2051 ATATGGCGGA CAAAGGCTAC CTCAAGCTCG GGCAGACCTC GATGCAGGGC  
 2101 ATCAAAGCCT ATATGCGGCA AAATCCGCAA CGCCTCGCCG AAGTTTTCGG  
 2151 TCAAAACCCC AGCTATATCT TTTTCCGCGA GCTTGCCGGA AGCAGCAATG  
 2201 ACGGTCCCGT CGGCGCACTG GGCACGCGGT TGATGGGGGA ATATGCCGGC  
 2251 GCAGTCGACC GGCACATAC TACCTTGGGC GCGCCCTTAT TTGTCGCCAC  
 2301 CGCCCATCCG GTTACCCGCA AAGCCCTCAA CCGCCTGATT ATGGCGCAGG  
 2351 ATACCGGCAG CGCGATTAAA GGCAGCGTGC GCGTGGATTA TTTTGGGGA  
 2401 TACGGCGACG AAGCCGCGCA ACTTGCCGGC AAACAGAAAA CCACGGGTTA  
 2451 CGTCTGGCAG CTCTACCCA ACGGTATGAA GCCCGAATAC CGCCCGTAAC  
 2501 TCGAG

1 MASPDVKSAD TLSKPAAPVV AEKETEVED APQAGSQGQG APSTQGSQDM  
 51 AAVSAENTGN GGAATTDDPK NEDEGPQNDM PQNSAESANQ TGNNQPADSS  
 101 DSAPASNAP ANGGSNFRV DLANGVLIDG PSQNTLTHC KGDSCNGDNL  
 151 LDEEAPSKSE FENLNESEI EKYKDKGSD KFTNLVATAV QANGTNKYVI  
 201 IYKDKSASS SARFRSARS RRLPAEMPL IPVNQADTLI VDGEAVSLTG  
 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKGEM LAGTAVYNGE  
 301 VLHFHTENGR PYPTRGRFAA KVDGSKSVD GIIDSGDDLH MGTQKFKAAI  
 351 DGNGFKGTWT ENGCGDVSGR FYGPAGEEVA GKYSYRPTDA EKGFGVFAG  
 401 KKEQDGSGGG GCQSKSIQTF PQPDTSVING PDRFVGIPDP AGTTVGGGGA  
 451 VYTVVPHLSL PHWAAQDFAK SLQSFRLGCA NLKNRQGWQD VCAQAFQTFV  
 501 HSFQAKQFFE RYFTPWQVAG NGSLAGTVTG YYEPVLKGDD RRTAQARFPI  
 551 YGIPDDFISV PLPAGLRSGK ALVRIRQTGK NSGTIDNTGG THTADLSRFP  
 601 ITARTTAIKG RFEGSRFLPY HTRNQINGGA LDGKAPILGY AEDPVELFFM  
 651 HIQGSGLRKT PSGKYIRIGY ADKNEHPYVS IGRYMADKGY LKLGQTSMQG  
 701 IKAYMRQNPQ RLAEVLQNP SYIFFRELGA SSNDGPVGLA GTPLMGEYAG  
 751 AVDRHYITLG APLFVATAHP VTRKALNRLI MAQDTGSAIK GAVRVDYFWG  
 801 YGDEAGELAG KQKTTGYVWQ LLENGMKPEY RP\*

**ΔG287-953**

1 ATGGCTAGCC CCGATGTAA ATCGGCGGAC ACGCTGTCAA AACCGGCCCGC  
 51 TCCTGTGTGT GCTGAAAAAG AGACAGAGGT AAAAGAAGAT CGGCCACAGG  
 101 CAGGTCTCTA AGGACAGGGC GCGCCATCCA CACAAGGCAG CCAAGATATG  
 151 GCGGCAGTTT CCGCAGAAAA TACAGGCAAT GCGGGTGGCG CAACAACGGA  
 201 CAAACCCAAA AATGAAGACG AGGACCGCA AAATGATATG CCGCAAAATT  
 251 CCGCCGAATC CGCAATCAA ACAGGGAACA ACCAACCCGC CGATTCTTCA  
 301 GATTCCGCCC CCGCGTCAA CCTGACACT GCGAATGGCG GTAGCAATTT  
 351 TGGAAAGGTT GATTGGCTA ATGGCGTTTT GATTGATGGG CCGTCGCAAA  
 401 ATATAACGTT GACCCACTGT AAAGCGGATT CTTGTAATGG TGATAATTTA  
 451 TTGGATGAAG AAGCACCGTC AAAATCAGAA TTTGAAAATT TAAATGAGTC  
 501 TGAACGAATT GAGAAATATA AGAAAGATGG GAAAAGCGAT AAATTTACTA  
 551 ATTTGGTTGC GACAGCAGTT CAAGCTAATG GAACTAACAA ATATGTCATC  
 601 ATTTATAAAG ACAAGTCCGC TTCATCTTCA TCTGCGCGAT TCAGGCGTTC  
 651 TGCACGGTCG AGGAGGTCG TTCCTGCCGA GATGCCGCTA ATCCCGTCA  
 701 ATCAGGCGGA TACGCTGATT GTCGATGGGG AAGCGGTGAG CCGTACGGGG  
 751 CATTCCGCGA ATATCTTCGC GCCCGAAGGG AATTACCGGT ATCTGACTTA

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801	CGGGGCGGAA	AAATTGCCCG	GCGGATCGTA	TGCCCTCCGT	GTGCAAGGCG
851	AACCGGCAAA	AGGCGAAATG	CTTGCTGGCA	CGGCCGTGTA	CAACGGCGAA
901	GTGCTGCATT	TTCATACGGA	AAACGGCCGT	CCGTACCCGA	CTAGAGGCAG
951	GTTTGCCGCA	AAAGTCGATT	TCGGCAGCAA	ATCTGTGGAC	GGCATTATCG
1001	ACAGCGGCGA	TGATTTGCA	ATGGGTACGC	AAAAATTCAA	AGCCGCCATC
1051	GATGGAAACG	GCTTTAAGGG	GA CT TGGACG	GAAAATGGCG	GCGGGGATGT
1101	TTCCGGAAGG	TTTACGGCC	CGGCCGGCGA	GGAAGTGGCG	GGAAAATACA
1151	GCTATCGCCC	GACAGATGCG	GAAAAGGGCG	GATTCCGGCGT	GTTTGC CGGC
1201	AAAAAAGAGC	AGGATGGATC	CGGAGGAGGA	GGAGCCACCT	ACAAAAGTGA
1251	CGAATATCAC	GCCAACGCCC	GTTTCGCCAT	CGACCATTTC	AACACCAGCA
1301	CCAACGTCGG	CGGTTTTTAC	GGTCTGACCG	GTTCCGTCGA	GTTCCGACCAA
1351	GCAAAACGCG	ACGGTAAAAT	CGACATCACC	ATCCCCGTTG	CCAACCTGCA
1401	AAGCGGTTTCG	CAACACTTTA	CCGACCACCT	GAAATCAGCC	GACATCTTCG
1451	ATGCCGCCCA	ATATCCGGAC	ATCCGCTTTG	TTTCCACCAA	ATCAACTTTC
1501	AACGGCAAAA	AAC TGGTTTC	CGTTGACGGC	AACCTGACCA	TGCACGGCAA
1551	AACCGCCCCC	GTCAAAC TCA	AAGCCGAAAA	ATTCAACTGC	TACCAAAGCC
1601	CGATGGCGAA	AACCGAAGTT	TGCGGCGGCG	ACTTCAGCAC	CACCATCGAC
1651	CGCACCAAAT	GGGGCGTGGA	CTACCTCGTT	AACGTTGGTA	TGACCAAAAG
1701	CGTCCGCATC	GACATCCAAA	TCGAGGCAGC	CAACAATAA	CTCGAG
1	MASPDVKSAD	TLSPAPFV	AEKETEVED	APQAGSQGG	APSTQGSQDM
51	AAVSAENTGN	GGAATTDKPK	NEDEGPQNDM	PQNSAESANQ	TGNQPADSS
101	DSAPASNAP	ANGGSNFRV	DLANGVLIDG	PSQNTLTHC	KGDSCNGDNL
151	LDEEAPSKSE	FENLNERI	EKYKDGKSD	KFTNLVATAV	QANGTNKYVI
201	IYKDSASSS	SARFRSARS	RRSLPAEMPL	IPVNOADTLI	VDGEAVSLTG
251	HSGNIFAPEG	NYRYLTGAE	KLPGGSYALR	VQGEPAKGEM	LAGTAVYNGE
301	VLHFTENGR	PYPTRGRFAA	KVDFGSKSVD	GIIDSGDDLH	MGTQKFKAAI
351	DGNFGKGTWT	ENGGGDVSGR	FYGPAGEEVA	GKYSYRPTDA	EKGFGVVFAG
401	KKEQDGS GGG	GATYKVD EYH	ANARFAIDHF	NTSTNVGGFY	GLTGSVEFDQ
451	AKRDGKIDIT	IPVANLQSGS	QHFTDHLKSA	DIFDAAQYPD	IRFVSTKFNF
501	NGKKLVSDG	NLTMHGKTAP	VKLKAEKFNC	YQSPMAKTEV	CGGDFSTTID
551	RTKWGV DYL V	NVGMTKSVRI	DIQIEAAKQ*		
<b>ΔG287-961</b>					
1	ATGGCTAGCC	CCGATGTTAA	ATCGGCGGAC	ACGCTGTCAA	AACCGGCCCGC
51	TCCTGTTGTT	GCTGAAAAAG	AGACAGAGGT	AAAAGAAGAT	GCGCCACAGG
101	CAGGTTCTCA	AGGACAGGGC	GCGCCATCCA	CACAAGGCAG	CCAAGATATG
151	GCGGCAGTTT	CGGCAGAAAA	TACAGGCAAT	GGCGGTGCGG	CAACAACGGA
201	CAAACCCAAA	AATGAAGACG	AGGGACCGCA	AAATGATATG	CCGCAAAATT
251	CCGCCGAATC	CGCAAATCAA	ACAGGGAACA	ACCAACCCGC	CGATTCTTCA
301	GATTCCGCCC	CCGCGTCAA	CCCTGCACCT	GCGAATGGCG	GTAGCAATTT
351	TGGAAGGGTT	GATTTGGCTA	ATGGCGTTT	GATTGATGGG	CGTCCGAAA
401	ATATAACGTT	GACCCACTGT	AAAGGCGATT	CTTGTAATGG	TGATAATTTA
451	TTGGATGAAG	AAGCACCGTC	AAAATCAGAA	TTTGAAAATT	TAAATGAGTC
501	TGAACGAATT	GAGAAATATA	AGAAAGATGG	GAAAAGCGAT	AAATTTACTA
551	ATTTGGTTGC	GACAGCAGTT	CAAGCTAATG	GAAC TAACAA	ATATGTCATC
601	ATTTATAAAG	ACAAGTCCGC	TTCATCTTCA	TCTGCGCGAT	TCAGGCGTTC
651	TGCACGGTCG	AGGAGGTCGC	TTCCTGCCGA	GATGCCGCTA	ATCCCCGTCA
701	ATCAGGCGGA	TACGCTGATT	GTCGATGGGG	AAGCGGTCAG	CCTGACGGGG
751	CATTCCGGCA	ATATCTTCGC	GCCCGAAGGG	AATTACCGGT	ATCTGACTTA
801	CGGGGCGGAA	AAATTGCCCG	GCGGATCGTA	TGCCCTCCGT	GTGCAAGGCG
851	AACCGGCAAA	AGGCGAAATG	CTTGCTGGCA	CGGCCGTGTA	CAACGGCGAA
901	GTGCTGCATT	TTCATACGGA	AAACGGCCGT	CCGTACCCGA	CTAGAGGCAG
951	GTTTGCCGCA	AAAGTCGATT	TCGGCAGCAA	ATCTGTGGAC	GGCATTATCG
1001	ACAGCGGCGA	TGATTTGCA	ATGGGTACGC	AAAAATTCAA	AGCCGCCATC
1051	GATGGAAACG	GCTTTAAGGG	GA CT TGGACG	GAAAATGGCG	GCGGGGATGT
1101	TTCCGGAAGG	TTTACGGCC	CGGCCGGCGA	GGAAGTGGCG	GGAAAATACA
1151	GCTATCGCCC	GACAGATGCG	GAAAAGGGCG	GATTCCGGCGT	GTTTGC CGGC
1201	AAAAAAGAGC	AGGATGGATC	CGGAGGAGGA	GGAGCCACAA	ACGACGACGA
1251	TGTTAAAAAA	GCTGCCACTG	TGGCCATTGC	TGCTGCCTAC	AACAATGGCC
1301	AAGAAATCAA	CGGTTTCAA	GCTGGAGAGA	CCATCTACGA	CATTGATGAA
1351	GACGGCACAA	TTACCAAAAA	AGACGCAACT	GCAGCCGATG	TTGAAGCCGA
1401	CGACTTTAAA	GGTCTGGGTC	TGAAAAAAGT	CGTGAATAAC	CTGACCAAAA
1451	CCGTCAATGA	AAACAAACAA	AACGTCGATG	CCAAAGTAAA	AGCTGCAGAA
1501	TCTGAAATAG	AAAAGTTAAC	AACCAAGTTA	GCAGACACTG	ATGCCGCTTT
1551	AGCAGATACT	GATGCCGCTC	TGGATGCAAC	CACCAACGCC	TTGAATAAAT

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1601 TGGGAGAAAA TATAACGACA TTTGCTGAAG AGACTAAGAC AAATATCGTA  
 1651 AAAATTGATG AAAAATTAGA AGCCGTGGCT GATACCGTCG ACAAGCATGC  
 1701 CGAAGCATTC AACGATATCG CCGATTCATT GGATGAAACC AACACTAAGG  
 1751 CAGACGAAGC CGTCAAAACC GCCAATGAAG CCAAACAGAC GGCCGAAGAA  
 1801 ACCAAACAAA ACGTCGATGC CAAAGTAAAA GCTGCAGAAA CTGCAGCAGG  
 1851 CAAAGCCGAA GCTGCCGCTG GCACAGCTAA TACTGCAGCC GACAAGGCCG  
 1901 AAGCTGTGCG TGCAAAAGTT ACCGACATCA AAGCTGATAT CGCTACGAAC  
 1951 AAAGATAATA TTGCTAAAAA AGCAAACAGT GCCGACGTGT ACACCAGAGA  
 2001 AGAGTCTGAC AGCAAAATTG TCAGAATTGA TGGTCTGAAC GCTACTACCG  
 2051 AAAAATTGGA CACACGCTTG GCTTCTGCTG AAAAATCCAT TGCCGATCAC  
 2101 GATACTCGCC TGAACGGTTT GGATAAAACA GTGTCAGACC TGCAGAAAGA  
 2151 AACC CGCAA GGCCTTCAG AACAAGCCGC GCTCTCCGGT CTGTTCCAAC  
 2201 CTTACAACGT GGGTCGGTTC AATGTAACGG CTGCAGTCGG CGGCTACAAA  
 2251 TCCGAATCGG CAGTCGCCAT CGGTACCGGC TTCCGCTTTA CCGAAACTTT  
 2301 TGCCGCCAAA GCAGGCGTGG CAGTCGGCAC TTCGTCCGGT TCTTCCGCAG  
 2351 CCTACCATGT CGGCGTCAAT TACGAGTGGT AACTCGAG  
  
 1 MASPDVKSAD TLSKPAAPVV AEKETEVRKED APQAGSQGG APSTQGSQDM  
 51 AAVSAENTGN GGAATTDKPK NEDEGPQNDM PQNSAESANQ TGNNQPADSS  
 101 DSAPASNPAP ANGGSNFRV DLANGVLIDG PSQNTLTHC KGDSCNGDNL  
 151 LDEEAPSKSE FENLNESEI EKYKKGKSD KFTNLVATAV QANGTNKYVI  
 201 IYKDKSASS SARFRSARS RRSLEPAEMPL IPVNQADTLI VDGEAVSLTG  
 251 HSGNIFAPEG NYRYLTYGAE KLPGGSYALR VQGEPAKGEM LAGTAVYNGE  
 301 VLHFHTENGR PYPTRGRFAA KVDGSGKSD GIIDSGDDLH MGTQKFKAAI  
 25 351 DGNGFKGTWT ENG GGVSGR FYGPAGEEVA GKYSYRPTDA EKG GFGVFAG  
 401 KKEQDGS GGG GATNDDVVK AATVAIAAAY NNGQEINGFK AGETIYDIDE  
 451 DGTITKKDAT AADVEADDFK GLGLKKVVTN LTKTVNENKQ NVDAKVKAAE  
 501 SEIEKLTTKL ADTDAALADT DAALDATTNA LNKLGENTIT FAEETKTNI  
 551 KIDEKLEAVA DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAE  
 30 601 TKQNVDAKVK AAETAAGKAE AAAGTANTAA DKAEVAARKV TDIKADIATN  
 651 KDNIKKANS ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH  
 701 DTRLNGLDKT VSDLRKETRQ GLAEQAALSG LFQPYNVGRF NVTAAVGGYK  
 751 SESAVAIGTG FRFTENFAAK AGVAVGTSSG SSAAYHGVN YEW\*

	ELISA	Bactericidal
ΔG287-953-His	3834	65536
ΔG287-961-His	108627	65536

35 The bactericidal efficacy (homologous strain) of antibodies raised against the hybrid proteins was compared with antibodies raised against simple mixtures of the component antigens (using 287-GST) for 919 and ORF46.1:

	Mixture with 287	Hybrid with ΔG287
919	32000	128000
ORF46.1	128	16000

Data for bactericidal activity against heterologous MenB strains and against serotypes A and C were also obtained:

	919		ORF46.1	
Strain	Mixture	Hybrid	Mixture	Hybrid
NGH38	1024	32000	-	16384
MC58	512	8192	-	512
BZ232	512	512	-	-
MenA (F6124)	512	32000	-	8192
MenC (C11)	>2048	>2048	-	-
MenC (BZ133)	>4096	64000	-	8192

The hybrid proteins with  $\Delta$ G287 at the N-terminus are therefore immunologically superior to simple mixtures, with  $\Delta$ G287-ORF46.1 being particularly effective, even against heterologous strains.  $\Delta$ G287-ORF46.1K may be expressed in pET-24b.

The same hybrid proteins were made using New Zealand strain 394/98 rather than 2996:

5	<b><u><math>\Delta</math>G287NZ-919</u></b>					
	1	ATGGCTAGCC	CCGATGTCAA	GTCGGCGGAC	ACGCTGTCAA	AACCTGCCGC
	51	CCCTGTTGTT	TCTGAAAAAG	AGACAGAGGC	AAAGGAAGAT	GCGCCACAGG
	101	CAGGTTCTCA	AGGACAGGGC	GCGCCATCCG	CACAAGGCGG	TCAAGATATG
	151	GCGGCGGTTT	CGGAAGAAAA	TACAGGCAAT	GGCGGTGCGG	CAGCAACGGA
10	201	CAAACCCAAA	AATGAAGACG	AGGGGGCGCA	AAATGATATG	CCGCAAAATG
	251	CCGCCGATAC	AGATAGTTTG	ACACCGAATC	ACACCCCGGC	TTCGAATATG
	301	CCGGCCGGAA	ATATGGAAAA	CCAAGCACCG	GATGCCGGGG	AATCGGAGCA
	351	GCCGGCAAAC	CAACCGGATA	TGGCAAATAC	GGCGGACGGA	ATGCAGGGTG
	401	ACGATCCGTC	GGCAGGCGGG	GAAATGCGG	GCAATACGGC	TGCCCAAGGT
15	451	ACAAATCAAG	CCGAAAACAA	TCAAACCGCC	GGTTCTCAA	ATCCTGCCTC
	501	TTCAACCAAT	CCTAGCGCCA	CGAATAGCGG	TGGTGATTTT	GGAAGGACGA
	551	ACGTGGGCAA	TTCTGTTGTG	ATTGACGGGC	CGTCGCAAAA	TATAACGTTG
	601	ACCCACTGTA	AAGGCGATTG	TTGTAGTGGC	AATAATTTCT	TGGATGAAGA
	651	AGTACAGCTA	AAATCAGAAT	TTGAAAAATT	AAGTGATGCA	GACAAAATAA
20	701	GTAATTACAA	GAAAGATGGG	AAGAATGACG	GGAAGAATGA	TAAATTTGTC
	751	GGTTTGGTTG	CCGATAGTGT	GCAGATGAAG	GGAATCAATC	AATATATTAT
	801	CTTTTATAAA	CCTAAACCCA	CTTCATTTGC	GCGATTTAGG	CGTTCTGCAC
	851	GGTCGAGGCG	GTCGCTTCCG	GCCGAGATGC	CGCTGATTCC	CGTCAATCAG
	901	GCGGATACGC	TGATTGTGCA	TGGGGAAGCG	GTCAGCCTGA	CGGGGCATTC
25	951	CGGCAATATC	TTGCGGCCCG	AAGGGAATTA	CCGGTATCTG	ACTTACGGGG
	1001	CGGAAAAATT	GCCCGGCGGA	TCGTATGCCC	TCCGTGTTCA	AGGCGAACCT
	1051	TCAAAAGGCG	AAATGCTCGC	GGGCACGGCA	GTGTACAACG	GCGAAGTGCT
	1101	GCATTTTCAT	ACGGAAAACG	GCCGTCCGTC	CCCGTCCAGA	GGCAGGTTTG
	1151	CCGCAAAAGT	CGATTTTCGGC	AGCAAATCTG	TGGACGGCAT	TATCGACAGC
30	1201	GGCGATGGTT	TGCATATGGG	TACGCAAAAA	TTCAAAGCCG	CCATCGATGG
	1251	AAACGGCTTT	AAGGGGACTT	GGACGGAAAA	TGGCGGCGGG	GATGTTTCCG
	1301	GAAAGTTTFA	CGGCCCGGCC	GGCGAGGAAG	TGGCGGGAAA	ATACAGCTAT
	1351	CGCCCAACAG	ATGCGGAAAA	GGGCGGATTG	GGCGTGTTTG	CCGGCAAAAA
	1401	AGAGCAGGAT	GGATCCGGAG	GAGGAGGATG	CCAAAGCAAG	AGCATCCAAA
35	1451	CCTTTCCGCA	ACCCGACACA	TCCGTCATCA	ACGGCCCGGA	CCGGCCGGTC
	1501	GGCATCCCCG	ACCCCGCCCG	AACGACGGTC	GGCGGCGGCG	GGGCCGTCTA
	1551	TACCGTTGTA	CCGCACCTGT	CCCTGCCCCA	CTGGGCGGCG	CAGGATTTCCG
	1601	CCAAAAGCCT	GCAATCCTTC	CGCCTCGGCT	GCGCCAATTT	GAAAAACCGC
	1651	CAAGGCTGGC	AGGATGTGTG	CGCCCAAGCC	TTTCAAACCC	CCGTCCATTC
40	1701	CTTTTACGGCA	AAACAGTTT	TTGAACGCTA	TTTACGCGCG	TGGCAGGTTG
	1751	CAGGCAACGG	AAGCCTTGCC	GGTACGGTTA	CCGGCTATTA	CGAGCCGGTG
	1801	CTGAAGGGCG	ACGACAGGCG	GACGGCACAA	GCCCGCTTCC	CGATTTACGG
	1851	TATTTCCGAC	GATTTTATCT	CCGTCCCCCT	GCCTGCCGGT	TTGCGGAGCG
	1901	GAAAAGCCCT	TGTCCGCATC	AGGCAGACGG	GAAAAACAG	CGGCACAATC
45	1951	GACAATACCG	GCGGCACACA	TACCGCCGAC	CTCTCCCGAT	TCCCCATCAC
	2001	CGCGCGCACA	ACGGCAATCA	AAGGCAGGTT	TGAAGGAAGC	CGTTCTCTCC

5	2051	CCTACCACAC	GCGCAACCAA	ATCAACGGCG	GCGCGCTTGA	CGGCAAAGCC
	2101	CCGATACTCG	GTTACGCCGA	AGACCCCGTC	GAACCTTTTT	TTATGCACAT
	2151	CCAAGGCTCG	GGCCGTCTGA	AAACCCCGTC	CGGCAAATAC	ATCCGCATCG
	2201	GCTATGCCGA	CAAAAACGAA	CATCCCTACG	TTTCCATCGG	ACGCTATATG
	2251	GCGGACAAAG	GCTACCTCAA	GCTCGGGCAG	ACCTCGATGC	AGGGCATCAA
	2301	AGCCTATATG	CGGCAAAATC	CGCAACGCCT	CGCCGAAGTT	TTGGGTCAAA
	2351	ACCCAGCTA	TATCTTTTTC	CGCGAGCTTG	CGCGAAGCAG	CAATGACGGT
	2401	CCCGTCGGCG	CACTGGGCAC	GCCGTTGATG	GGGGAATATG	CCGGCGCAGT
	2451	CGACCGGCAC	TACATTACCT	TGGGCGCGCC	CTTATTTGTC	GCCACCGCCC
	10	2501	ATCCGGTTAC	CCGCAAAGCC	CTCAACCGCC	TGATTATGGC
2551	GGCAGCGCGA	TTAAAGGCGC	GGTGCGCGTG	GATTATTTTT	GGGGATACGG	
2601	CGACGAAGCC	GGCGAACTTG	CCGGCAAACA	GAAAACCACG	GGTTACGTCT	
2651	GGCAGCTCCT	ACCCAACGGT	ATGAAGCCCG	AATACCGCCC	GTAAAAGCTT	
20	1	MASPDVKSAD	TLSPAAPV	SEKETEAKED	APQAGSQGG	APSAQGGQDM
	51	AAVSEBENTGN	GGAAATDKPK	NEDEGAQNDM	PQNAADTDSL	TPNHTPASNM
	101	PAGNMENQAP	DAGESEQPAN	QPDMAANTADG	MQGDDPSAGG	ENAGNTAAQG
	151	TNQAEENQTA	GSQNPASSTN	PSATNSGGDF	GRTNVGNSVV	IDGPSQNTFL
	201	THCKGDSCSG	NNFLDEEVQL	KSEFEKLSDA	DKISNYKKDG	KNDGKNDKFF
	251	GLVADSVQMK	GINQYIIFYK	PKPTSFARFR	RSARSRRSLP	AEMPLIPVNO
	301	ADTLIVDGEA	VSLTGHSGNI	FAPEGNYRYL	TYGAELPQG	SYALRVQGEF
	351	SKGEMLAGTA	VYNGEVLHFF	TENGRPSPSR	GRFAAKVDFG	SKSVDGIIDS
	401	GDGLHMGTOK	FKAADGNNGF	KGTWTENGGG	DVSGKFYFGA	GEEVAGKYSY
	25	451	RPTDAEKGGF	GVFAGKKEQD	GSGGGGCGSK	SIQTFPQPD
501	GIPDPAGTTV	GGGGAIVTVV	PHLSLPHWAA	QDFAKSLQSF	RLGCANLKNR	
551	QGWQDVCAQA	FQTFVHSFQA	KQFFERYFTP	WQVAGNGSLA	GTVTGYEYEPV	
601	LKGDRTTAQ	ARFPIYGIPD	DFISVPLPAG	LRSGKALVRI	RQTGKNSGTI	
651	DNTGGTHTAD	LSRFPITART	TAIKGRFEFS	RFLPYHTNRQ	INGGALDGKA	
30	701	PILGYAEDPV	ELFFMHIQGS	GRLKTPSGKY	IRIGYADKNE	HPYVSIGRYM
751	ADKGYLKLQ	TSMQGIKAYM	RQNPORLAEV	LGQNPYSIFF	RELAGSSNDG	
801	PVGALGTPLM	GEYAGAVDRH	YITLGAPLFV	ATAHPVTRKA	LNRLIMAQDT	
851	GSAIKGAVRV	DYFWGYGDEA	GELAGKQKTT	GYVWQLLPNG	MKPEYRP*	
35	<u>ΔG287NZ-953</u>					
1	ATGGCTAGCC	CCGATGTCAA	GTGCGCGGAC	ACGCTGTCAA	AACCTGCCGC	
51	CCCTGTTGTT	TCTGAAAAAG	AGACAGAGGC	AAAGGAAGAT	GCGCCACAGG	
101	CAGGTTCTCA	AGGACAGGGC	GCGCCATCCG	CACAAGGCGG	TCAAGATATG	
151	GCGGCGGTTT	CGGAAGAAAA	TACAGGCAAT	GGCGGTGCGG	CAGCAACGGA	
40	201	CAAACCCAAA	AATGAAGACG	AGGGGGCGCA	AAATGATATG	CCGCAAAATG
251	CCGCCGATAC	AGATAGTTTG	ACACCGAATC	ACACCCCGGC	TTCGAATATG	
301	CCGGCCGGA	ATATGGAAAA	CCAAGCACCG	GATGCCGGGG	AATCGGAGCA	
351	GCCGGCAAAC	CAACCGGATA	TGGCAAATAC	GGCGGACGGA	ATGCAGGGTG	
401	ACGATCCGTC	GGCAGGCGGG	GAAAATGCCG	GCAATACGGC	TGCCCCAAGGT	
45	451	ACAAATCAAG	CCGAAAACAA	TCAAACCGCC	GGTTCTCAA	ATCCTGCCTC
501	TTCAACCAAT	CCTAGCGCCA	CGAATAGCGG	TGGTGATTTT	GGAAGGACGA	
551	ACGTGGGCAA	TTCTGTTGTG	ATTGACGGGC	CGTCGCAAAA	TATAACGTTG	
601	ACCCACTGTA	AAGGCGATTC	TTGTAGTGGC	AATAATTTCT	TGGATGAAGA	
651	AGTACAGCTA	AAATCAGAAT	TTGAAAAATT	AAGTGATGCA	GACAAAATAA	
50	701	GTAATTACAA	GAAAGATGGG	AAGAATGACG	GGAAGAATGA	TAAATTTGTC
751	GGTTTGGTTG	CCGATAGTGT	GCAGATGAAG	GGAATCAATC	AATATATTAT	
801	CTTTTATAAA	CCTAAACCCA	CTTCATTTGC	GCGATTTAGG	CGTTCGTCAC	
851	GGTCGAGGCG	GTGCTTCCG	GCCGAGATGC	CGCTGATTCC	CGTCAATCAG	
901	CGGGATACGC	TGATTGTGCA	TGGGGAAGCG	GTCAGCTTCA	CGGGGCATTC	
55	951	CGGCAATATC	TTCGCGCCCG	AAGGGAATTA	CCGGTATCTG	ACTTACGGGG
1001	CGGAAAAATT	GCCCGGCGGA	TCGTATGCCC	TCCGTGTTCA	AGGCGAACCT	
1051	TCAAAGGCG	AAATGCTCGC	GGGCACGGCA	GTGTACAACG	GCGAAGTGCT	
1101	GCATTTTCAT	ACGGAAGACG	GCCGTCCGTC	CCCGTCCAGA	GGCAGGTTTG	
1151	CCGCAAAAGT	CGATTTCCGG	AGCAAACTGT	TGGACGGCAT	TATCGACAGC	
60	1201	GGCGATGGTT	TGCATATGGG	TACGCAAAAA	TTCAAAGCCG	CCATCGATGG
1251	AAACGGCTTT	AAGGGGACTT	GGACGGAAAA	TGGCGGCGGG	GATGTTTCCG	
1301	GAAAGTTTTA	CGGCCCGGCC	GGCGAGGAAG	TGGCGGGAAA	ATACAGCTAT	
1351	CGCCAACAG	ATGCGGAAAA	GGGCGGATTC	GGCGTGTTTG	CCGGCAAAAA	
1401	AGAGCAGGAT	GGATCCGGAG	GAGGAGGAGC	CACCTACAAA	GTGGACGAAT	
65	1451	ATCACGCCAA	CGCCCGTTTC	GCCATCGACC	ATTTCAACAC	CAGCACCAAC
1501	GTCGGCGGTT	TTTACGGTCT	GACCGGTTCC	GTCGAGTTTC	ACCAAGCAAA	
1551	ACGCGACGGT	AAAATCGACA	TCACCATCCC	CGTTGCCAAC	CTGCAAAGCG	

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5	1601	GTTCGCAACA	CTTTACCGAC	CACCTGAAAT	CAGCCGACAT	CTTCGATGCC
	1651	GCCCAATATC	CGGACATCCG	CTTTGTTTCC	ACCAAAATCA	ACTTCAACGG
	1701	CAAAAACTG	GTTTCCGTTG	ACGGCAACCT	GACCATGCAC	GGCAAAACCG
	1751	CCCCCGTCAA	ACTCAAAGCC	GAAAAATTCA	ACTGCTACCA	AAGCCCCGATG
	1801	GCGAAAACCG	AAGTTTGC GG	CGGCGACTTC	AGCACCAACA	TCGACCGCAC
	1851	CAAATGGGGC	GTGGACTACC	TCGTTAACGT	TGGTATGACC	AAAAGCGTCC
	1901	GCATCGACAT	CCAAATCGAG	GCAGCCAAAC	AATAAAAGCT	T
10	1	MASPDVKSAD	TLSPKPAAPVV	SEKETEAKED	APQAGSQGQG	APSAQGGQDM
	51	AAVSEENTGN	GGAAATDKPK	NEDEGAQNDM	PQNAADTDSL	TPNHTPASNM
	101	PAGNMENQAP	DAGESEQPAN	QPDMAATADG	MQGDDPSAGG	ENAGNTAAQG
	151	TNQAENNQTA	GSQNPASSTN	PSATNSGGDF	GRTNVGNSVV	IDGPSQNTIL
	201	THCKGDS CSG	NNFLDEEVQL	KSEFEKLSDA	DKISNYKKDG	KNDGKNDKFV
15	251	GLVADSVQMK	GINQYIIFYK	PKPTS FARFR	RSARSRRSLP	AEMPLIPVNO
	301	ADTLIVDGEA	VSLTGHSGNI	FAPEGNYRYL	TYGAELP PGG	SYALRVQGEF
	351	SKGEMLAGTA	VYNGEVLHFF	TENGRPSPSR	GRFAAKVDFG	SKSVDGIIDS
	401	GDGLHMG TQK	FKAAIDGNF	KGTWTENG GG	DVSGKFY GPA	GEEVAGKYSY
	451	RPTDAERGGF	GVFAGKEQD	GSGGGGATYK	VDEYHANARF	AIDHFNSTSN
20	501	VGGFYGLTGS	VEFDQAKRDG	KIDITIPVAN	LQSGSQHFTD	HLKSADIFDA
	551	AQYPDIRFVS	TKFNFNGKKL	VSVDGNLTMH	GKTAPVKLKA	EKFNCYQSPM
	601	AKTEVCGGDF	STTIDRTKWG	VDYLVNVGMT	KSVRIDIQIE	AAKQ*
25	<u>AG287NZ-961</u>					
	1	ATGGCTAGCC	CCGATGTCAA	GTCGGCGGAC	ACGCTGTCAA	AACCTGCCGC
	51	CCCTGTTGTT	TCTGAAAAAG	AGACAGAGGC	AAAGGAAGAT	GCGCCACAGG
	101	CAGGTTCTCA	AGGACAGGGC	GCGCCATCCG	CACAAGCGCG	TCAAGATATG
	151	GCGGCGGTTT	CGGAAGAAAA	TACAGGCAAT	GGCGGTGCGG	CAGCAACGGA
30	201	CAAACCCAAA	AATGAAGACG	AGGGGGCGCA	AAATGATATG	CCGCAAAATG
	251	CCGCCGATAC	AGATAGTTTG	ACACCGAATC	ACACCCCGGC	TTCGAATATG
	301	CCGGCCGAAA	ATATGGAAAA	CCAAGCACCG	GATGCCGGGG	AATCGGAGCA
	351	GCCGGCAAAC	CAACCGGATA	TGGCAAATAC	GGCGGACGGA	ATGCAGGGTG
	401	ACGATCCGTC	GGCAGGCGGG	GAAAATGCCG	GCAATACGGC	TGCCCAAGGT
35	451	ACAAATCAAG	CCGAAAAACAA	TCAAACCGCC	GGTTCTCAAA	ATCCTGCCTC
	501	TTCAACCAAT	CCTAGCGCCA	CGAATAGCGG	TGGTGATTTT	GGAAGGACGA
	551	ACGTGGGCAA	TTCTGTTGTG	ATTGACGGGC	CGTCGCAAAA	TATAACGTTG
	601	ACCCACTGTA	AAGGCGATTG	TTGTAGTGCC	AATAATTTCT	TGGATGAAGA
	651	AGTACAGCTA	AAATCAGAAT	TTGAAAAATT	AAGTGTATGCA	GACAAAAATA
40	701	GTAATTACAA	GAAAGATGGG	AAGAATGACG	GGAAGAATGA	TAAATTTGTC
	751	GGTTTGTTTG	CCGATAGTGT	GCAGATGAAG	GGAATCAATG	AATATATTAT
	801	CTTTTATAAA	CCTAAACCCA	CTTCATTTCG	GCGATTAGG	CGTTCTGCAC
	851	GGTCGAGGCG	GTCGCTTCCG	GCCGAGATGC	CGCTGATTCC	CGTCAATCAG
	901	GCGGATACGC	TGATTGT CGA	TGGGGAAGCG	GTCAGCCTGA	CGGGGCATTG
45	951	CGGCAATATC	TTCGCGCCCG	AAGGGAATTA	CCGGTATCTG	ACTTACGGGG
	1001	CGGAAAAATT	GCCCGGCGGA	TCGTATGCCC	TCCGTGTTCA	AGGCGAACCT
	1051	TCAAAAGGCG	AAATGCTCGC	GGGCACGGCA	GTGTACAACG	GCGAAGTGCT
	1101	GCATTTTCAT	ACGGAAAACG	GCCGTCCGTC	CCCGTCCAGA	GGCAGGTTTG
	1151	CCGCAAAAGT	CGATTTCCGG	AGCAAATCTG	TGGACGGCAT	TATCGACAGC
50	1201	GGCGATGGTT	TGCATATGGG	TACGCAAAAA	TTCAAAGCCG	CCATCGATGG
	1251	AAACGGCTTT	AAGGGGACTT	GGACGGAAAA	TGGCGGCGGG	GATGTTTCCG
	1301	GAAAGTTTTA	CGGCCCGGCC	GGCGAGGAAG	TGGCGGGAAG	ATACAGCTAT
	1351	CGCCCAACAG	ATGCGGAAAA	GGGCGGATTG	GGCGTGTTTG	CCGCAAAAAA
	1401	AGAGCAGGAT	GGATCCGGAG	GAGGAGGAGC	CACAAACGAC	GACGATGTTA
55	1451	AAAAAGCTGC	CACTGTGGCC	ATTGCTGCTG	CCTACAACAA	TGGCCAAGAA
	1501	ATCAACGGTT	TCAAAGCTGG	AGAGACCATC	TACGACATTG	ATGAAGACGG
	1551	CACAATTACC	AAAAAAGACG	CAACTGCAGC	CGATGTTGAA	GCCGACGACT
	1601	TTAAAGGTCT	GGGTCTGAAA	AAAGTCGTGA	CTAACCTGAC	CAAAACCGTC
	1651	AATGAAAAACA	AACAAAACGT	CGATGCCAAA	GTAAGAGCTG	CAGAATCTGA
60	1701	AATAGAAAAG	TTAACAACCA	AGTTAGCAGA	CACTGATGCC	GCTTTAGCAG
	1751	ATACTGATGC	CGTCTGGAT	GCAACCACCA	ACGCCTTGAA	TAAATTGGGA
	1801	GAAAAATATA	CGACATTTGC	TGAAGAGACT	AAGACAAAATA	TCGTAAAAAT
	1851	TGATGAAAAA	TTAGAAGCCG	TGGCTGATAC	CGTCGACAAG	CATGCCGAAG
	1901	CATTCAACGA	TATCGCCGAT	TCATTGGATG	AAACCAACAC	TAAGGCAGAC
65	1951	GAAGCCGTCA	AAACCGCCAA	TGAAGCCAAA	CAGACGGCCG	AAGAAACCAA
	2001	ACAAAACGTC	GATGCCAAAG	TAAAAGCTGC	AGAAACTGCA	GCAGGCAAAG
	2051	CCGAAGCTGC	CGCTGGCACA	GCTAATACTG	CAGCCGACAA	GGCCGAAGCT
	2101	GTCGCTGCAA	AAGTTACCGA	CATCAAAGCT	GATATCGCTA	CGAACAAAGA

2151 TAATATTGCT AAAAAAGCAA ACAGTGCCGA CGTGACACC AGAGAAGAGT  
 2201 CTGACAGCAA ATTTGTCAGA ATTGATGGTC TGAACGCTAC TACCGAAAAA  
 2251 TTGGACACAC GCTTGGCTTC TGCTGAAAAA TCCATTGCCG ATCACGATAC  
 2301 TCGCCTGAAC GGTTTGGATA AAACAGTGTC AGACCTGCGC AAAGAAACCC  
 2351 GCCAAGGCCT TGCAGAACAA GCCGCGCTCT CCGGTCTGTT CCAACCTTAC  
 2401 AACGTGGGTC GGTTCAATGT AACGGCTGCA GTCGGCGGCT ACAAATCCGA  
 2451 ATCGGCAGTC GCCATCGGTA CCGGCTTCCG CTTTACCGAA AACTTTGCCG  
 2501 CCAAAGCAGG CGTGGCAGTC GGCACCTCGT CCGGTTCTTC CGCAGCCTAC  
 2551 CATGTCGGCG TCAATTACGA GTGGTAAAAG CTT

1 MASPDVKSAD TLSKPAAPVV SEKETEAKED APQAGSQGG APSAQGGQDM  
 51 AAVSEENTGN GGAAATDKPK NEDEGAQNDM PQNAADTDSL TPNHTPASNM  
 101 PAGNMENQAP DAGESEQPAN QPDMANTADG MQGDDPSAGG ENAGNTAAQG  
 151 TNQAENNQTA GSQNPASSTN PSATNSGGDF GRTNVGNSVV IDGPSQMITL  
 201 THCKGDSCSG NNFLDEEVQL KSEFEKLSDA DKISNYKKDG KNDGKNDKFFV  
 251 GLVADSVQMK GINQYIIFYK PKPTSFAFR RSARSRRSLP AEMPLIPVNVQ  
 301 ADTLIVDGEA VSLTGHSGNI FAPEGNYRYL TYGAEKLP GG SYALRVQGEF  
 351 SKGEMLAGTA VYNGEVLHFH TENGRPSPSR GRFAAKVDFG SKSVDGIIDS  
 401 GDGLHMGTK FKAIDGNF KGTWTENG GG DVSGKFYGP GEEVAGKYSY  
 451 RPTDAEKGGF GVFAKGKEQD GSGGGGATND DDVKKAAATVA IAAAYNNGQE  
 501 INGFKAGETI YDIDEDGTIT KKDATAADVE ADDFKGLGLK KVVTNLTKTIV  
 551 NENKQNVDAK VKAAESEIEK LTTKLADTDA ALADTDAALD ATTNALNKLK  
 601 ENITTFAEET KTNIVKIDEK LEAVADTVDK HAEAFNDIAD SLDETNTKAD  
 651 EAVKTANEAK QTAEETKQNV DAKVKAETA AGKAEAAAGT ANTAADKAEA  
 701 VAAKVTDIKA DIATNMKNIA KKANSADVYT REEDSKFVR IDGLNATTEK  
 751 LDTRLASAEK SIADHDTRLN GLDKTVSDLR KETROGLAEQ AALSGLFQPY  
 801 NVGRFNVTA VGGYKSESAV AIGTGFRFTE NFAAKAGVAV GTSSGSSAAY  
 851 HVGVNYEW\*

### 30 *ΔG983 and hybrids*

Bactericidal titres generated in response to  $\Delta$ G983 (His-fusion) were measured against various strains, including the homologous 2996 strain:

	2996	NGH38	BZ133
$\Delta$ G983	512	128	128

$\Delta$ G983 was also expressed as a hybrid, with ORF46.1, 741, 961 or 961c at its C-terminus:

$\Delta$ G983-ORF46.1

35 1 ATGACTTCTG CGCCCGACTT CAATGCAGGC GGTACCGGTA TCGGCAGCAA  
 51 CAGCAGAGCA ACAACAGCGA AATCAGCAGC AGTATCTTAC GCCCGTATCA  
 101 AGAACGAAAT GTGCAAAGAC AGAAGCATGC TCTGTGCCGG TCGGGATGAC  
 151 GTTGC GGTTA CAGACAGGGA TGCCAAAATC AATGCCCCCC CCCCGAATCT  
 201 GCATACCGGA GACTTTCCAA ACCCAAATGA CGCATACAAG AATTGTGATCA  
 40 251 ACCTCAAACC TGCAATTGAA GCAGGCTATA CAGGACGCGG GGTAGAGGTA  
 301 GGTATCGTCG ACACAGGCGA ATCCGTCGGC AGCATATCCT TTCCCGAACT  
 351 GTATGGCAGA AAAGAACACG GCTATAACGA AAATTACAAA AACTATACGG  
 401 CGTATATGCG GAAGGAAGCG CCTGAAGACG GAGGCGGTAA AGACATTGAA  
 451 GCTTCTTTCG ACGATGAGGC CGTTATAGAG ACTGAAGCAA AGCCGACGGA  
 45 501 TATCCGCCAC GTAAAAGAAA TCGGACACAT CGATTTGGTC TCCCATATTA  
 551 TTGGCGGGCG TTCCGTGGAC GGCAGACCTG CAGGCGGTAT TGCGCCCGAT  
 601 GCGACGCTAC ACATAATGAA TACGAATGAT GAAACCAAGA ACGAAATGAT  
 651 GGTTCAGCC ATCCGCAATG CATGGGTCAA GCTGGGCGAA CGTGGCGTGC  
 701 GCATCGTCAA TAACAGTTT GGAACAACAT CGAGGGCAGG CACTGCCGAC  
 50 751 CTTTTCAAA TAGCCAATT GGAGGAGCAG TACCGCCAAG CGTTGCTCGA  
 801 CTATTCCGGC GGTGATAAAA CAGACGAGG TATCCGCTG ATGCAACAGA  
 851 GCGATTACGG CAACCTGTCC TACCACATCC GTAATAAAAA CATGCTTTTC  
 901 ATCTTTTCGA CAGGCAATGA CGCACAAGCT CAGCCCAACA CATATGCCCT  
 951 ATTGCCATTT TATGAAAAAG ACGCTCAAAA AGGCATTATC ACAGTCGCAG  
 55 1001 GCGTAGACCG CAGTGGAGAA AAGTTCAAAC GGGAAATGTA TGGAGAACCG  
 1051 GGTACAGAAC CGTTGAGTA TGGCTCCAAC CATTGCGGAA TTACTGCCAT  
 1101 GTGGTGCCCTG TCGGCACCCCT ATGAAGCAAG CGTCCGTTTC ACCCGTACAA

	1151	ACCCGATTCA	AATTGCCGGA	ACATCCTTTT	CCGCACCCAT	CGTAACCGGC
	1201	ACGGCGGCTC	TGCTGCTGCA	GAAATACCCG	TGGATGAGCA	ACGACAACCT
	1251	GCGTACCACG	TTGCTGACGA	CGGCTCAGGA	CATCGGTGCA	GTCGGCGTGG
5	1301	ACAGCAAGTT	CGGCTGGGGA	CTGCTGGATG	CGGGTAAGGC	CATGAACGGA
	1351	CCCGCGTCCT	TTCCGTTCGG	CGACTTTTACC	GCCGATACGA	AAGGTACATC
	1401	CGATATTGCC	TACTCCTTCC	GTAACGACAT	TTCAGGCACG	GGCGGCCTGA
	1451	TCAAAAAAGG	CGGCAGCCAA	CTGCAACTGC	ACGGCAACAA	CACCTATACG
	1501	GGCAAAACCA	TTATCGAAGG	CGGTTCGCTG	GTGTTGTACG	GCAACAACAA
10	1551	ATCGGATATG	CGCGTCGAAA	CCAAAGGTGC	GCTGATTTAT	AACGGGGCGG
	1601	CATCCGGCGG	CAGCCTGAAC	AGCGACGGCA	TTGTCTATCT	GGCAGATACC
	1651	GACCAATCCG	GCGCAACCGA	AACCGTACAC	ATCAAAGGCA	GTCTGCAGCT
	1701	GGACGGCAAA	GGTACGCTGT	ACACACGTTT	GGGCAAACTG	CTGAAAGTGG
	1751	ACGGTACGGC	GATTATCGGC	GGCAAGCTGT	ACATGTCGGC	ACGCGGCAAG
15	1801	GGGGCAGGCT	ATCTCAACAG	TACCGGACGA	CGTGTTCCCT	TCTGTAGTGC
	1851	CGCCAAAATC	GGGCAGGATT	ATTCTTTCTT	CACAAACATC	GAAACCGACG
	1901	GCGGCCTGCT	GGCTTCCCTC	GACAGCGTCG	AAAAAACAGC	GGGCAGTGAA
	1951	GGCGACACGC	TGTCTTATTA	TGTCCGTCGC	GGCAATGCGG	CACGGACTGC
	2001	TTCGGCAGCG	GCACATTCCG	CGCCCGCCGG	TCTGAAACAC	GCCGTAGAAC
20	2051	AGGGCGGCAG	CAATCTGGAA	AACCTGATGG	TCGAACTGGA	TGCCTCCGAA
	2101	TCATCCGCAA	CACCCGAGAC	GGTTGAAACT	GCGGCAGCCG	ACCGCAGAGA
	2151	TATGCCGGGC	ATCCGCCCCCT	ACGGCGCAAC	TTTCCGCGCA	GCGGCAGCCG
	2201	TACAGCATGC	GAATGCCGCC	GACGGGTGAC	GCATCTTCAA	CAGTCTCGCC
	2251	GCTACCGTCT	ATGCCGACAG	TACCGCCGCC	CATGCCGATA	TGCAAGGACG
25	2301	CCGCTGAAA	GCCGTATCCG	ACGGGTGGA	CCACAACGGC	ACGGGTCTGC
	2351	GCGTCATCGC	GCAAAACCAA	CAGGACGGTG	GAACGTGGGA	ACAGGGCGGT
	2401	GTTGAAGGCA	AAATGCGCGG	CAGTACCCAA	ACCGTCGGCA	TTGCCGCGAA
	2451	AACCGGCGAA	AATACGACAG	CAGCCGCCAC	ACTGGGCATG	GGACGCAGCA
	2501	CATGGAGCGA	AAACAGTGCA	AATGCAAAAA	CCGACAGCAT	TAGTCTGTTT
30	2551	GCAGGCATAC	GGCAGCATGC	GGGCGATATC	GGCTATCTCA	AAGGCCTGTT
	2601	CTCCTACGGA	CGCTACAAAA	ACAGCATCAG	CCGCAGCACC	GGTGCAGACG
	2651	AACATGCGGA	AGGCAGCGTC	AACGGCACGC	TGATGCAGCT	GGGCGCACTG
	2701	GGCGGTGTCA	ACGTTCCGTT	TGCCGCAACG	GGAGATTTGA	CGGTCTGAAGG
	2751	CGGTCTGCGC	TACGACCTGC	TCAAACAGGA	TGCATTGCGC	GAAAAAGGCA
35	2801	GTGCTTTGGG	CTGGAGCGGC	AACAGCCTCA	CTGAAGGCAC	GCTGGTCGGA
	2851	CTCGCGGGTC	TGAAGCTGTC	GCAACCCTTG	AGCGATAAAG	CCGTCTGTGT
	2901	TGCAACGGCG	GGCGTGGAAC	GCGACCTGAA	CGGACGCGAC	TACACGGTAA
	2951	GCGCGGGCTT	TACCGCGCGG	ACTGCAGCAA	CCGGCAAGAC	GGGGCGACGC
	3001	AATATGCCGC	ACACCCGTCT	GGTTGCCGGC	CTGGGCGCGG	ATGTCGAATT
40	3051	CGGCAACGGC	TGGAACGGCT	TGGCACGTTA	CAGCTACGCC	GGTTCCAAAC
	3101	AGTACGGCAA	CCACAGCGGA	CGAGTCGGCG	TAGGCTACCG	GTTCCTCGAC
	3151	GGTGGCGGAG	GCACTGGATC	CTCAGATTTG	GCAAACGATT	CTTTTATCCG
	3201	GCAGGTTCTC	GACCGTCAGC	ATTTGGAACC	CGACGGGAAA	TACCACCTAT
	3251	TCGCGAGCAG	GGGGGAACCT	GCCGAGCGCA	GCGGCCATAT	CGGATTGGGA
45	3301	AAAATACAAA	GCCATCAGTT	GGGCAACCTG	ATGATTCAAC	AGGCGGCCAT
	3351	TAAAGGAAAT	ATCGGCTACA	TTGTCCGCTT	TTCCGATCAC	GGGCACGAAG
	3401	TCCATTCCCC	CTTCGACAAC	CATGCCTCAC	ATTCCGATTG	TGATGAAGCC
	3451	GGTAGTCCCG	TTGACGGATT	TAGCCTTTAC	CGCATCCATT	GGGACGGATA
	3501	CGAACACCAT	CCCGCCGACG	GCTATGACGG	GCCACAGGGC	GGCGGCTATC
50	3551	CCGCTCCCAA	AGGCGCGAGG	GATATATACA	GCTACGACAT	AAAAGGCGTT
	3601	GCCCAAAATA	TCCGCCTCAA	CCTGACCGAC	AACCGCAGCA	CCGGACAACG
	3651	GCTTGCCGAC	CGTTTCCACA	ATGCCGGTAG	TATGCTGACG	CAAGGAGTAG
	3701	GCGACGGATT	CAAACGCGCC	ACCCGATACA	GCCCCGAGCT	GGACAGATCG
	3751	GGCAATGCCG	CCGAAGCCTT	CAACGGCACT	GCAGATATCG	TTAAAAACAT
55	3801	CATCGGCGCG	GCAGGAGAAA	TTGTCGGCGC	AGGCGATGCC	GTGCAGGGCA
	3851	TAAAGCAAGG	CTCAAACATT	GCTGTATGTC	ACGGCTTGGG	TCTGCTTTCC
	3901	ACCGAAAACA	AGATGGCGCG	CATCAACGAT	TTGGCAGATA	TGGCGCAACT
	3951	CAAAGACTAT	GCCGCAGCAG	CCATCCGCGA	TTGGGCAGTC	CAAAACCCCA
	4001	ATGCCGCACA	AGGCATAGAA	GCCGTCAGCA	ATATCTTTAT	GGCAGCCATC
60	4051	CCCATCAAAG	GGATTGGAGC	TGTTCCGGGA	AAATACGGCT	TGGGCGGCAT
	4101	CACGGCACAT	CCTATCAAGC	GGTCGCAGAT	GGGCGCGATC	GCATTGCCGA
	4151	AAGGGAAATC	CGCCGTCAGC	GACAAATTTG	CCGATGCGGC	ATACGCCAAA
	4201	TACCCGTCCC	CTTACCATTG	CCGAAATATC	CGTTCAAACCT	TGGAGCAGCG
	4251	TTACGGCAAA	GAAAACATCA	CCTCCTCAAC	CGTGCCGCGG	TCAAACGGCA
65	4301	AAAATGTCAA	ACTGGCAGAC	CAACGCCACC	CGAAGACAGG	CGTACCGTTT
	4351	GACGGTAAAG	GGTTTCCGAA	TTTGTGAGAAG	CACGTGAAAT	ATGATACGCT
	4401	CGAGCACCAC	CACCACCACC	ACTGA		

1 MTSAPDFNAG GTGIGSNSRA TTAKSAAVSY AGIKNEMCKD RSMCLCAGRDD  
 51 VAVTDRDAKI NAPPNNLHTG DFPNPNDAYK NLINLKPAIE AGYTGRGVEV  
 101 GIVDTGESVG SISFPPELYGR KEHGYNENYK NYTAYMRKEA PEDGGGKDIE  
 15 151 ASFDDEAVIE TEAKPTDIRH VKEIGHIDL V SHIIGGRSVD GRPAGGIAPD  
 201 ATLHIMNTND ETKNEMVAA IRNAWVKLGE RGVRIVNSNF GTTSRAGTAD  
 251 LFQIANSEEQ YRQALLDYSG GDKTDEGIRL MQQSDYGNLS YHIRNKNMLF  
 301 IFSTGNDAQA QPNTYALLPF YEKDAQKGII TVAGVDRSGE KFKREMYGEP  
 351 GTEPLEYGSN HCGITAMWCL SAPYEASVRF TRTNPIQIAG TSFSAPIVTG  
 10 401 TAALLLQKYP WMSNDNLRTT LLTTAQDIGA VGVDSKFGWG LLDAGKAMNG  
 451 PASFPFGDFT ADTKGTS DIA YSFRNDISGT GGLIKKGGSQ LQLHGNNTYT  
 501 GKTIIEGGS L VLYGNKSDM RVETK GALIY NGAASGGS LN SDGIVYLADT  
 551 DQSGANETVH IKGSLQLDGK GTLYTRLGKL LKVDGTAIIG GKLYMSARGK  
 601 GAGYLNSTGR RVPFLSAAKI GQDYSFFTNI ETDGGLLASL DSVEKTAGSE  
 15 651 GDTLSYYVRR GNAARTASAA AHSAPAGLKH AVEQGSNLE NLMVELDASE  
 701 SSATPETVET AAADRTDMPG IRPYGATFRA AAAVQHANA DGVRIFNSLA  
 751 ATVYADSTAA HADMQRRLK AVSDGLDHNG TGLRVIAQTQ QDGGTWEQGG  
 801 VEGKMRGSTQ TVGIAAKTGE NTTAAATLGM GRSTWSENSA NAKTDSISLF  
 851 AGIRHDAGDI GYLKGLFSYG RYKNSISRST GADEHAEGSV NGTLMQLGAL  
 20 901 GGVNVPFAAT GDLTVEGGLR YDLLKQDAFA EKGSALGWSG NSLTEGTLVG  
 951 LAGLKSQPL SDKAVLFATA GVERDLNGRD YTVTGGFTGA TAATGKTGAR  
 1001 NMPHTRLVAG LGADVEFGNG WNGLARYSYA GSKQYGNHSG RVGVGYRFLD  
 1051 GGGGTGSSDL ANDSFIRQVL DRQHFE PDGK YHLFGSRGEL AERSGHIGLG  
 1101 KIQSHQLGNL MIQQA AIKGN IGYIVRFS DH GHEVHSPFDN HASHSDSDEA  
 1151 GSPVDGFSLY RIHWDDGYEHH PADGYDGPQG GGYPA PKGAR DIYSYDIKGV  
 25 1201 AQNIRLNLTD NRSTGQRLAD RFHNAGSMLT QGVGDGFKRA TRYSPELDRS  
 1251 GNAAEAFNGT ADIVKNIIGA AGEIVGAGDA VQGISSEGSNI AVMHGLGLLS  
 1301 TENKMARIND LADMAQLKDY AAAAIRDWAV QNPNA AQGIE AVSNIFMAAI  
 1351 PIKGIGAVRG KYGLGGITAH PIKRSQMGAI ALPKGKSAVS DNFADAAYAK  
 1401 YPSPYHSRNI RSNLEQRYGK ENITSSTVPP SNGKNVKLAD QRHPKTGVPE  
 30 1451 DGKGFNF EK HVKYDTLEHH HHHH\*

AG983-741

1 ATGACTTCTG CGCCCGACTT CAATGCAGGC GGTACCGGTA TCGGCAGCAA  
 35 51 CAGCAGAGCA ACAACAGCGA AATCAGCAGC AGTATCTTAC GCCGGTATCA  
 101 AGAACGAAAT GTGCAAAGAC AGAAGCATGC TCTGTGCCGG TCGGGATGAC  
 151 GTTGCGGTTA CAGACAGGGA TGCCAAAATC AATGCCCCCC CCCCGAATCT  
 201 GCATACCGGA GACTTTCCAA ACCCAAATGA CGCATACAAG AATTTGATCA  
 40 251 ACCTCAAACC TGCAATTGAA GCAGGCTATA CAGGACGCGG GGTAGAGGTA  
 301 GGTATCGTCG ACACAGGCGA ATCCGTCGGC AGCATATCCT TTCCCGAACT  
 351 GTATGGCAGA AAAGAACACG GCTATAACGA AAATTACAAA AACTATACGG  
 401 CGTATATGCG GAAGGAAGCG CCTGAAGACG GAGGCGGTAA AGACATTGAA  
 451 GCTTCTTTTCG ACGATGAGGC CGTTATAGAG ACTGAAGCAA AGCCGACGGA  
 501 TATCCGCCAC GTAAAAGAAA TCGGACACAT CGATTTGTC TCCCATATTA  
 45 551 TTGGCGGGCG TTCCGTGGAC GGCAGACCTG CAGGCGGTAT TGCGCCCGAT  
 601 GCGACGCTAC ACATAATGAA TACGAATGAT GAAACCAAGA ACGAAATGAT  
 651 GGTTCGAGCC ATCCGCAATG CATGGGTCAA GCTGGGCGAA CGTGGCGTGC  
 701 GCATCGTCAA TAACAGTTTT GGAACAACAT CGAGGGCAGG CACTGCCGAC  
 751 CTTTTC AAA TAGCCAATTC GGAGGAGCAG TACCGCCAAG CGTTGCTCGA  
 50 801 CTATTCCGGC GGTGATAAAA CAGACGAGGG TATCCGCCTG ATGCAACAGA  
 851 GCGATTACGG CAACCTGTCC TACCACATCC GTAATAAAAA CATGCTTTTC  
 901 ATCTTTTCGA CAGGCAATGA CGCACAAGCT CAGCCCAACA CATATGCCCT  
 951 ATTGCCATTT TATGAAAAAG ACGCTCAAAA AGGCATTATC ACAGTCGCAG  
 1001 GCGTAGACCG CAGTGGAGAA AAGTTCAAAC GGGAAATGTA TGGAGAACCG  
 55 1051 GGTACAGAAC CGCTTGAGTA TGGCTCCAAC CATTGCGGAA TTAGTGCCAT  
 1101 GTGGTGCTTG TCGGCACCTT ATGAAGCAAG CGTCCGTTTC ACCCGTACAA  
 1151 ACCCGATTCA AATTGCCGGA ACATCCTTTT CCGCACCCAT CGTAACCGGC  
 1201 ACGCGGCTC TGCTGCTGCA GAAATACCGG TGGATGAGCA ACGACAACCT  
 1251 GCGTACCACG TTGCTGACGA CGGCTCAGGA CATCGGTGCA GTCGGCGTGG  
 60 1301 ACAGCAAGTT CGGCTGGGGA CTGCTGGATG CGGGTAAGGC CATGAACGGA  
 1351 CCCGCTCCT TTCCGTTCCG CGACTTTACC GCCGATACGA AAGGTACATC  
 1401 CGATATTGCC TACTCCTTCC GTAACGACAT TTCAGGCACG GCGCGCTGA  
 1451 TCAAAAAAGG CGGCAGCCAA CTGCAACTGC ACGGCAACAA CACCTATACG  
 1501 GGCAAAACCA TTATCGAAGG CGGTTTCGCTG GTGTTGTACG GCAACAACAA  
 65 1551 ATCGGATATG CGCGTCGAAA CCAAAGGTGC GCTGATTTAT AACGGGCGG  
 1601 CATCCGGCGG CAGCCTGAAC AGCGACGCGA TTGTCTATCT GGCAGATACC  
 1651 GACCAATCCG GCGCAAACGA AACC GTACAC ATCAAAGGCA GTCTGCAGCT

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	1701	GGACGGCAAA	GGTACGCTGT	ACACACGTTT	GGGCAAACGTG	CTGAAAGTGG
	1751	ACGGTACGGC	GATTATCGGC	GGCAAGCTGT	ACATGTTCGGC	ACGCGGCAAG
	1801	GGGGCAGGCT	ATCTCAACAG	TACCGGACGA	CGTGTTCCTT	TCCTGAGTGC
5	1851	CGCCAAATC	GGGCAGGATT	ATTCTTTCTT	CACAAACATC	GAAACCGACG
	1901	GCGGCCCTGCT	GGCTTCCCTC	GACAGCGTCG	AAAAAACAGC	GGGCAGTGAA
	1951	GGCGACACGC	TGTCTTATTA	TGTCCGTCGC	GGCAATGCGG	CACGGACTGC
	2001	TTCGGCAGCG	GCACATTCCG	CGCCCGCCGG	TCTGAAACAC	CCCGTAGAAC
	2051	AGGGCGGCAG	CAATCTGGAA	AACCTGATGG	TCGAACTGGA	TGCCTCCGAA
10	2101	TCATCCGCAA	CACCCGAGAC	GGTTGAAACT	GCGGCAGCCG	ACCGCACAGA
	2151	TATGCCGGGC	ATCCGCCCCCT	ACGGCGCAAC	TTTCCGCGCA	GCGGCAGCCG
	2201	TACAGCATGC	GAATGCCGCC	GACGGTGTAC	GCATCTTCAA	CAGTCTCGCC
	2251	GCTACCGTCT	ATGCCGACAG	TACCGCCGCC	CATGCCGATA	TGCAGGGACG
	2301	CCGCCGTGAAA	GCCGTATCGG	ACGGGTGGA	CCACAACGGC	ACGGGTCTGC
15	2351	GCGTCAATCGC	GCAAAACCAA	CAGGACGGTG	GAACGTGGGA	ACAGGGCCGGT
	2401	GTTGAAGGCA	AAATGCGCGG	CAGTACCCAA	ACCGTCGGCA	TTGCCGCGAA
	2451	AACCGGCGAA	AATACGACAG	CAGCCGCCAC	ACTGGGCATG	GGACGCAGCA
	2501	CATGGAGCGA	AAACAGTGCA	AATGCAAAAA	CCGACAGCAT	TAGTCTGTTT
	2551	GCAGGCATAC	GGCAGCATGC	GGGCGATATC	GGCTATCTCA	AAGGCCTGTT
20	2601	CTCCTACGGA	CGCTACAAAA	ACAGCATCAG	CCGCAGCACC	GGTGCAGGAC
	2651	AACATGCGGA	AGGCAGCGTC	AACGGCACGC	TGATGCAGCT	GGGGCAGCTG
	2701	GGCGGTGTCA	ACGTTCCGTT	TGCCGCAACG	GGAGATTTGA	CGGTGCAAGG
	2751	CGGTCTGCGC	TACGACCTGC	TCAAACAGGA	TGCATTGCGC	GAAAAAGGCA
	2801	GTGCTTTGGG	CTGGAGCGGC	AACAGCCTCA	CTGAAGGCAC	GCTGGTCGGA
25	2851	CTCGCGGGTC	TGAAGCTGTC	GCAACCCTTG	AGCGATAAAG	CCGTCTCTGT
	2901	TGCAACGGCG	GGCGTGGAA	GCGACCTGAA	CGGACGCGAC	TACACGGTAA
	2951	CGGGCGGCTT	TACCGCGCGC	ACTGCAGCAA	CCGGCAAGAC	GGGGCAGCGC
	3001	AATATGCCGC	ACACCCGTCT	GGTTGCCGGC	CTGGGCGCGG	ATGTCGAATT
	3051	CGGCAACGGC	TGGAACGGCT	TGGCACGTTA	CAGCTACGCC	GGTTCCAAAC
30	3101	AGTACGGCAA	CCACAGCGGA	CGAGTCGGCG	TAGGCTACCG	GTTCTCTGAG
	3151	GGATCCGGAG	GGGGTGGTGT	CGCCGCCGAC	ATCGGTGCGG	GGCTTGCCGA
	3201	TGCACTAACC	GCACCGCTCG	ACCATAAAGA	CAAAGGTTTG	CAGTCTTTGA
	3251	CGCTGGATCA	GTCCGTCAGG	AAAAACGAGA	AACTGAAAGT	GGCGGCACAA
	3301	GGTGCAGAAA	AAACTTATGG	AAACGGTGAC	AGCCTCAATA	CGGGCAAATT
35	3351	GAAGAACGAC	AAGGTCAGCC	GTTTCGACTT	TATCCGCCAA	ATCGAAGTGG
	3401	ACGGGCAGCT	CATTACCTTG	GAGAGTGGAG	AGTTCCAGT	ATACAAACAA
	3451	AGCCATTCCG	CCTTAACCGC	CTTTCAGACC	GAGCAAATAC	AAGATTGCGA
	3501	GCATTCCGGG	AAGATGGTTG	CGAAACGCCA	GTTTCAGAAAT	GGCGGCATAG
	3551	CGGGCGAACA	TACATCTTTT	GACAAGCTTC	CCGAAGGCGG	CAGGGCGACA
40	3601	TATCGCGGGA	CGGCGTTCGG	TTCAGACGAT	GCCGGCGGAA	AACTGACCTA
	3651	CACCATAGAT	TTCGCCGCCA	AGCAGGGAAA	CGGCAAAATC	GAACATTTGA
	3701	AATCGCCAGA	ACTCAATGTC	GACCTGGCCG	CCGCCGATAT	CAAGCCGGAT
	3751	GGAAAACGCC	ATGCCGTCAT	CAGCGGTTCC	GTCCTTTTACA	ACCAAGCCGA
	3801	GAAAGGCAGT	TACTCCCTCG	GTATCTTTGG	CGGAAAAGCC	CAGGAAGTTG
45	3851	CCGGCAGCGC	GGAAGTGAAA	ACCGTAAACG	GCATACGCCA	TATCGGCCCTT
	3901	GCCGCCAAGC	AACTCGAGCA	CCACCACCAC	CACCACTGA	
	1	MTSAPDFNAG	GTGIGSNSRA	TTAKSAAVSY	AGIKNEMCKD	RSMLCAGRDD
	51	VAVTDRDAKI	NAPPPNLHTG	DFPNPNDAKY	NLINLKPAIE	AGYTGRGVEV
50	101	GIVDTGESVG	SISFPELYGR	KEHGYNENYK	NYTAYMRKEA	PEDGGGKDIE
	151	ASFDDEAVIE	TEAKPTDIRH	VKEIGHIDL	SHIIGGRSVD	GRPAGGIAPD
	201	ATLHIMNTND	ETKNEMMVAA	IRNAWVKLGE	RGVRIVNNSF	GTTSRAGTAD
	251	LFQIANSEEQ	YRQALLDYS	GDKTDEGIRL	MQQSDYGNLS	YHIRNKNMLF
	301	IFSTGNDQAQ	QPNYALLPF	YEKDAQKGII	TVAGVDRSGE	KFKREMYGEP
55	351	GTEPLEYGSN	HCGITAMWCL	SAPYEASVRF	TRTNPIQIAG	TSFSAPIVTG
	401	TAALLLQKYP	WMSNDNLRTT	LLTTAQDIGA	VGVDSKFGWG	LLDAGKAMNG
	451	PASFPPGDFT	ADTKGTSDIA	YSFRNDISGT	GGLIKKGGSQ	LQLHGNNTYT
	501	GKTIIEGSSL	VLYGNKSDM	RVETKGALII	NGAASGGS LN	SDGIVYLADT
	551	DQSGANETVH	IKGSLQLD	GTLYTRLGKL	LKVDGTALIG	GKLYMSARGK
60	601	GAGYLNSTGR	RVFPLSAAKI	GQDYSFFTNI	ETDGGLLASL	DSVEKTAGSE
	651	GDTLSYVRR	GNAARTASAA	AHSAPAGLKH	AVEQGSNLE	NLMVELDASE
	701	SSATPETVET	AAADRTDMPG	IRPYGATFRA	AAAVQHANA	DGVRIFNSLA
	751	ATVYADSTAA	HADMQRRLK	AVSDGLDHNG	TGLRVIAQTQ	QDGGTWEQGG
	801	VEGKMRGSTQ	TVGIAAKTGE	NTTAAATLGM	GRSTWSENSA	NAKTDSISLF
65	851	AGIRHDAGDI	GYLKGLFSYG	RYKNSISRST	GADEHAEGSV	NGTLMQLGAL
	901	GGVNVPPFAAT	GDLTVEGGLR	YDLLKQDAFA	EKGSALGWSG	NSLTBEGTLVG
	951	LAGLKLSQLP	SDKAVLFATA	GVERDLNGRD	YTVTGFTGA	TAATGKTGAR
	1001	NMPHTRLVAG	LGADVEFGNG	WNGLARYSYA	GSKQYGNHSG	RVGVGYRFLE

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1051 GSGGGGVAAD IGAGLADALT APLDHKDKGL QSLTLDQSVR KNEKLLKAAQ  
 1101 GAEKTYGNBD SLNTGKLNKD KVSRLFDFIRQ IEVDGQLITL ESGEFQVYKQ  
 1151 SHSALTAFQT BQIQDSEHSG KMKVAKRQFRI GDIAGEHTSF DKLPEGGRAT  
 1201 YRGTAFGSDD AGGKLTFTTID FAAKQNGNKI EHLKSPELNV DLAAADIKPD  
 1251 GKRHAIVISGS VLYNQAEKGS YSLGIFGGKA QEVAGSAEVK TVNGIRHIGL  
 1301 AAKQLEHHHH HH\*

AG983-961

10 1 ATGACTTCTG CGCCCGACTT CAATGCAGGC GGTACCGGTA TCGGCAGCAA  
 51 CAGCAGAGCA ACAACAGCGA AATCAGCAGC AGTATCTTAC GCCGGTATCA  
 101 AGAACGAAAT GTGCAAAGAC AGAAGCATGC TCTGTGCCGG TCGGGATGAC  
 151 GTTGCGGTTA CAGACAGGGA TGCCAAAATC AATGCCCCCC CCCCGAATCT  
 201 GCATACCGGA GACTTTCCAA ACCCAAATGA CGCATACAAG AATTTGATCA  
 15 251 ACCTCAAACC TGCAATTGAA GCAGGCTATA CAGGACGCGG GGTAGAGGTA  
 301 GGTATCGTCG ACACAGGCGA ATCCGTCGGC AGCATATCCT TTCCCGAACT  
 351 GTATGGCAGA AAAGAACACG GCTATAACGA AAATTACAAA AACTATACGG  
 401 CGTATATGCG GAAGGAAGCG CCTGAAGACG GAGGCGGTAA AGACATTGAA  
 451 GCTTCTTTTCG ACGATGAGGC CGTTATAGAG ACTGAAGCAA AGCCGACGGA  
 20 501 TATCCGCCAC GTAAAAGAAA TCGGACACAT CGATTTGGTC TCCCATATTA  
 551 TTGGCGGGCG TTCCGTGGAC GGCAGACCTG CAGGCGGTAT TCGCGCCGAT  
 601 GCGACGCTAC ACATAATGAA TACGAATGAT GAAACCAAGA ACGAAATGAT  
 651 GGTTCAGGCC ATCCGCAATG CATGGGTCAA GCTGGGCGAA CGTGGCGTGC  
 701 GCATCGTCAA TAACAGTTT GGAACAACAT CGAGGGCAGG CACTGCCGAC  
 25 751 CTTTTCCAAA TAGCCAATTC GGAGGAGCAG TACCGCCAAG CGTTGCTCGA  
 801 CTATTCCGGC GGTGATAAAA CAGACGAGGG TATCCGCTTG ATGCAACAGA  
 851 GCGATTACGG CAACCTGTCC TACCACATCC GTAATAAAAA CATGCTTTTC  
 901 ATCTTTTCGA CAGGCAATGA CGCACAAGCT CAGCCCAACA CATATGCCCT  
 951 APTGCCATTT TATGAAAAAG ACGCTCAAAA AGGCATTATC ACAGTCGCAG  
 30 1001 GCGTAGACCG CAGTGGAGAA AAGTTCAAAC GGGAAATGTA TGGAGAACCG  
 1051 GGTACAGAAC CGCTTGAGTA TGGCTCCAAC CATTGCGGAA TTAATGCCAT  
 1101 GTGGTGCCTG TCGGCACCCT ATGAAGCAAG CGTCCGTTTC ACCCGTACAA  
 1151 ACCCGATTCA AATTGCCGGA ACATCCTTTT CCGCACCCAT CGTAACCGGC  
 1201 ACGGCGGCTC TGCTGTGCA GAAATACCCG TGATGAGCA ACGACAACCT  
 35 1251 GCGTACCACG TTGCTGACGA CGGCTCAGGA CATCGGTGCA GTCGGCGTGG  
 1301 ACAGCAAGTT CGGCTGGGAG CTGCTGGATG CGGGTAAGGC CATGAACGGA  
 1351 CCGCGTCTCT TTCCGTTCCG CGACTTTACC GCCGATACGA AAGGTACATC  
 1401 CGATATTGCC TACTCCTTCC GTAACGACAT TTCAGGCACG GCGGCTGTA  
 1451 TCAAAAAAGG CGGCAGCCAA CTGCAACTGC ACGGCAACAA CACCTATACG  
 40 1501 GGCAAAACCA TTATCGAAGG CGGTTCGCTG GTGTTGTACG GCAACAACAA  
 1551 ATCGGATATG CGCGTCGAAA CCAAAGGTGC GCTGATTTAT AACGGGGCGG  
 1601 CATCCGGCGG CAGCCTGAAC AGCGACGGCA TTGTCTATCT GGCAGATACC  
 1651 GACCAATCCG GCGCAACAGA AACCGTACAC ATCAAAGGCA GTCTGCAGCT  
 45 1701 GGACGGCAAA GGTACGCTGT ACACACGTTT GGGCAAACTG CTGAAAGTGG  
 1751 ACGGTACGGC GATTATCGGC GGCAAGCTGT ACATGTCGGC ACGCGGCAAG  
 1801 GGGGCAGGCT ATCTCAACAG TACCGGACGA CGTGTTCCCT TCCTGAGTGC  
 1851 CGCCAAAATC GGGCAGGATT ATTCTTTCTT CACAAAATC GAAACCGACG  
 1901 CGGCGCTGCT GGCTTCCCTC GACAGCGTCG AAAAAACAGC GGGCAGTGAA  
 1951 GCGGACACGC TGTCTTATTA TGTCCGTCGC GGCAATGCGG CACGGACTGC  
 50 2001 TTCGGCAGCG GCACATTCCG CGCCCGCCGG TCTGAAACAC GCCGTAGAAC  
 2051 AGGGCGGCAG CAATCTGGAA AACCTGATGG TCGAACTGGA TGCCTCCGAA  
 2101 TCATCCGCAA CACCCGAGAC GGTGAAACT GCGGCAGCCG ACCGCACAGA  
 2151 TATGCCGGGC ATCCGCCCCC ACGGCGCAAC TTTCCGCGCA GCGGCAGCCG  
 2201 TACAGCATGC GAATCCCGCT GACGGTGTAC GCATCTTCAA CAGTCTCGCC  
 55 2251 GCTACCGTCT ATGCCGACAG TACCGCCGCC CATGCCGATA TGCAGGACG  
 2301 CCGCCTGAAA GCCGTATCGG ACGGGTTGGA CCACAACGGC ACGGGTCTGC  
 2351 GCGTCATCGC GCAAACCCAA CAGGACGGTG GAACGTGGGA ACAGGGCGGT  
 2401 GTTGAAGGCA AAATGCGCGG CAGTACCCAA ACCGTCCGCA TTGCCCGGAA  
 2451 AACC GGCGAA AATACGACAG CAGCCGCCAC ACTGGGCATG GGACGCAGCA  
 60 2501 CATGGAGCGA AAACAGTGCA AATGCAAAAA CCGACAGCAT TAGTCTGTTT  
 2551 GCAGGCATAC GGCACGATGC GGGCGATATC GGCTATCTCA AAGGCCGTGT  
 2601 CTCCTACGGA CGCTACAAAA ACAGCATCAG CCGCAGCACC GGTGCGGACG  
 2651 AACATGCGGA AGGCAGCGTC AACGGCACGC TGATGCAGCT GGGCGCACTG  
 2701 GCGGGTGTCA ACGTTCCGTT TGCCGCAACG GGAGATTTGA CGGTGCAAGG  
 65 2751 CGGTCTGCGC TACGACCTGC TCAAACAGGA TGCATTGCGC GAAAAAGGCA  
 2801 GTGCTTTGGG CTGGAGCGGC AACAGCCTCA CTGAAGGCAC GCTGGTCGGA  
 2851 CTCGCGGGTC TGAAGCTGTC GCAACCCCTG ACGGATAAAG CCGTCTGTTT

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2901	TGCAACGGCG	GGCGTGGAAC	GCGACCTGAA	CGGACGCGAC	TACACGGTAA
2951	CGGGCGGCTT	TACCGGCGCG	ACTGCAGCAA	CCGCAAGAC	GGGGGCACGC
3001	AATATGCCGC	ACACCCGTCT	GGTTGCCCGC	CTGGGCGCGG	ATGTCTGAATT
3051	CGGCAACGGC	TGGAACGGCT	TGGCACGTTA	CAGCTACGCC	GGTTCCAAAC
3101	AGTACGGCAA	CCACAGCGGA	CGAGTCGGCG	TAGGCTACCG	GTTCCTCGAG
3151	GGTGGCGGAG	GCACTGGATC	CGCCACAAAC	GACGACGATG	TTAAAAAAGC
3201	TGCCACTGTG	GCCATTGCTG	CTGCCCTACAA	CAATGGCCAA	GAAATCAACG
3251	GTTTCAAAGC	TGGAGAGACC	ATCTACGACA	TTGATGAAGA	CGGCACAAAT
3301	ACCAAAAAAG	ACGCAACTGC	AGCCGATGTT	GAAGCCGACG	ACTTTAAAGG
3351	TCTGGGTCTG	AAAAAAGTCG	TGACTAACCT	GACCAAAACC	GTCAATGAAA
3401	ACAAACAAAA	CGTCGATGCC	AAAGTAAAAG	CTGCAGAATC	TGAAATAGAA
3451	AAGTTAACAA	CCAAGTTAGC	AGACACTGAT	GCCGCTTTAG	CAGATACTGA
3501	TGCCGCTCTG	GATGCAACCA	CCAACGCCTT	GAATAAATTG	GGAGAAAATA
3551	TAACGACATT	TGCTGAAGAG	ACTAAGACAA	ATATCGTAAA	AATTGTGAA
3601	AAATTAGAAG	CCGTGGCTGA	TACCGTCGAC	AAGCATGCCG	AAGCATTCAA
3651	CGATATCGCC	GATTCAATTG	ATGAAACCAA	CACTAAGGCA	GACGAAGCCG
3701	TCAAAACCGC	CAATGAAGCC	AAACAGACGG	CCGAAGAAAC	CAAAACAAAC
3751	GTCGATGCCA	AAGTAAAAGC	TGCAGAAACT	GCAGCAGGCA	AAGCCGAAGC
3801	TGCCGCTGGC	ACAGCTAATA	CTGCAGCCGA	CAAGGCCGAA	GCTGTGCGTG
3851	CAAAAGTTAC	CGACATCAAA	GCTGATATCG	CTACGAACAA	AGATAATATT
3901	GCTAAAAAAG	CAAAACAGTC	CGACGTGTAC	ACCAGAGAAG	AGTCTGACAG
3951	CAAATTTGTC	AGAATTGATG	GTCTGAACGC	TACTACCGAA	AAATTGGACA
4001	CACGCTTGGC	TTCTGCTGAA	AAATCCATTG	CCGATCACGA	TACTCGCCTG
4051	AACGGTTTGG	ATAAAACAGT	GTCAGACCTG	CGCAAAGAAA	CCCGCCAAGG
4101	CCTTGCAGAA	CAAGCCGCGC	TCTCCGGTCT	GTTCCAACCT	TACAACGTGG
4151	TCCGGTTCAA	TGTAACGGCT	GCAGTCGGCG	GCTACAAATC	CGAATCGGCA
4201	GTCGCCATCG	GTACCGGCTT	CCGCTTTACC	GAAAACTTTG	CCGCCAAAGC
4251	AGGCGTGGCA	GTCGGCACTT	CGTCCGGTTC	TTCCGCAGCC	TACCATGTCTG
4301	GCGTCAATTA	CGAGTGGCTC	GAGCACCACC	ACCACCACCA	CTGA
1	MTSAPDFNAG	GTGIGSNSRA	TTAKSAAVS	AGIKNEMCKD	RSMLCAGRDD
51	VAVTDRDAKI	NAPPPNLHTG	DFPNPNDAYK	NLNLKPAIE	AGYTGRGVFV
101	GIVDTGESVG	SISFPELYGR	KEHGYNENYK	NYTAYMRKEA	PEDGGGKDIE
151	ASFDDAEVIE	TEAKPTDIRH	VKEIGHIDL	SHIIGGRSVD	GRPAGGIAPD
201	ATLHIMNTND	ETKNEMMVAA	IRNAWVKLGE	RGVRIVNNF	GTTSRAGTAD
251	LFQIANSEEQ	YRQALLDYS	GDKTDEGIRL	MQQSDYGNLS	YHIRKNMFL
301	IFSTGNDAQA	QNTYALLPF	YEKDAQKGI	TVAGVDRSGE	KFKREMYGEP
351	GTEPLEYGSN	HCGITAMWCL	SAPYEASVRF	TRTNPIQIAG	TFSAPIVTIG
401	TAALLLQKYP	WMSNDNLRTT	LLTTAQDIGA	VGVDSKFGWG	LLDAGKAMNG
451	PASFPFGDFT	ADTKGTS DIA	YSFRNDISGT	GGLIKKGGSQ	LQLHGNNTYT
501	GKTIIEGSSL	VLYGNKSDM	RVETKGALIY	NGAASGGSLN	SDGIVYLADT
551	DQSGANETVH	IKGSLQLDGK	GTLYTRLGKL	LKVDGTAIIG	GKLYMSARGK
601	GAGYLNSTGR	RVPFLSAAKI	GQDYSFFTNI	ETDGGLLASL	DSVEKTAGSE
651	GDTLSYVVR	GNAARTASAA	AHSAPAGLKH	AVEQGGSNLE	NLMVELDASE
701	SSATPETVET	AAADRTDMPG	IRPYGATFRA	AAAVQHANA	DGVRIFNLSA
751	ATVYADSTAA	HADMQRRLK	AVSDGLDHNG	TGLRVIAQTQ	QDGGTWEQGG
801	VEGKMRGSTQ	TVGIAAKTGE	NTTAAATLGM	GRSTWSENSA	NAKTDSISLF
851	AGIRHDAGDI	GYLKGLFSYG	RYKNSISRST	GADEHAEGSV	NGTLMQLGAL
901	GCVNVPFAAT	GDLTVEGGLR	YDLLKQDAFA	EKGSA LGWSG	NSLTEGTLVG
951	LAGLKLSQLP	SDKAVLFATA	GVERDLNGRD	YTVTGGFTGA	TAATGKTGAR
1001	NMPHTRLVAG	LGADVEFGNG	WNGLARYSYA	GSKQYGNHSG	RVGVGYRFL
1051	GGGGTGSATN	DDDVKKAAATV	AIAAAYNNQ	EINGFKAGET	IYDIDEDGTI
1101	TKKDATAADV	EADDFKGLGL	KKVVTNLTKT	VNENKQNVDA	KVKAASEIE
1151	KLTTKLADTD	AALADTDAL	DATNALNKL	GENITTFAGE	TKTNIVKIDE
1201	KLEAVADTV	KHAEAFNDIA	DSLDETNTKA	DEAVKTANEA	KQTAETKQN
1251	VDAKVKAET	AAGKAEAAAG	TANTAADKAE	AVAAKVTDIK	ADIATNKDNI
1301	AKKANSADV	TREESDSKFV	RIDGLNATTE	KLDTRLASAE	KSIADHDTRL
1351	NGLDKTVSDL	RKETRQGLAE	QAALSGLFQP	YNVGRFNVTA	AVGGYKSESA
1401	VAIGTGFRFT	ENFAAKAGVA	VGTSSGSSAA	YHGVNIEWL	EHHHHHH*

**AG983-961c**

1	ATGACTTCTG	CGCCCCACTT	CAATGCAGGC	GGTACCGGTA	TCGGCAGCAA
51	CAGCAGAGCA	ACAACAGCGA	AATCAGCAGC	AGTATCTTAC	GCCGGTATCA
101	AGAACGAAAT	GTGCAAGAC	AGAAGCATGC	TCTGTGCCCG	TCGGGATGAC
151	GTTGCGGTTA	CAGACAGGGA	TGCCAAAATC	AATGCCCCCC	CCCCGAATCT
201	GCATACCGGA	GACTTTCCAA	ACCCAAATGA	CGCATACAAG	AATTTGATCA

251	ACCTCAAACC	TGCAATTGAA	GCAGGCTATA	CAGGACGCGG	GGTAGAGGTA
301	GGTATCGTCG	ACACAGGCGA	ATCCGTCGCG	AGCATATCCT	TTCCCGAACT
351	GTATGGCAGA	AAAGAACACG	GCTATAACGA	AAATTACAAA	AACTATACGG
401	CGTATATGCG	GAAGGAAGCG	CCTGAAGACG	GAGGCGGTAA	AGACATTGAA
451	GCTTCTTTTCG	ACGATGAGGC	CGTTATAGAG	ACTGAAAGCAA	AGCCGACGGA
501	TATCCGCCAC	GTAAAAGAAA	TCGGACACAT	CGATTTGGTC	TCCCATATTA
551	TTGGCGGGCG	TTCCGTGGAC	GGCAGACCTG	CAGGCGGTAT	TGCGCCCGAT
601	GCGACGCTAC	ACATAATGAA	TACGAATGAT	GAAACCAAGA	ACGAAATGAT
651	GGTTGCAGCC	ATCCGCAATG	CATGGGTCAA	GCTGGGCGAA	CGTGGCGTGC
701	GCATCGTCAA	TAACAGTTTT	GGAACAACAT	CGAGGGCAGG	CACTGCCGAC
751	CTTTTCCAAA	TAGCCAATTC	GGAGGAGCAG	TACCGCCAAG	CGTTGCTCGA
801	CTATTCCGGC	GGTGATAAAA	CAGACGAGGG	TATCCGCCTG	ATGCAACAGA
851	GCGATTACGG	CAACCTGTCC	TACCACATCC	GTAATAAAAA	CATGCTTTTC
901	ATCTTTTCGA	CAGGCAATGA	CGCACAAAGCT	CAGCCCAACA	CATATGCCCT
951	ATTGCCATTT	TATGAAAAAG	ACGCTCAAAA	AGGCATTATC	ACAGTCGCAG
1001	GCGTAGACCG	CAGTGGAGAA	AAGTTCAAAC	GGGAAATGTA	TGGAGAACCG
1051	GGTACAGAAC	CGCTTGAGTA	TGGCTCCAAC	CATTGCGGAA	TTACTGCCAT
1101	GTGGTGCCTG	TCGGCACCCCT	ATGAAGCAAG	CGTCCGTTTC	ACCCGTACAA
1151	ACCGATTCA	AATTGCCGGA	ACATCCTTTT	CCGCACCCAT	CGTAACCGG
1201	ACGCGGGCTC	TCGTGCTGCA	GAAATACCCG	TGGATGAGCA	ACGACAACCT
1251	GCGTACCACG	TTGCTGACGA	CGGCTCAGGA	CATCGGTGCA	GTCGGCGTGG
1301	ACAGCAAGTT	CGGCTGGGGA	CTGCTGGATG	CGGGTAAGGC	CATGAACGGA
1351	CCCGCGTCTT	TTCCGTTCGG	CGACTTTACC	GCCGATACGA	AAGGTACATC
1401	CGATATTGCC	TACTCCTTCC	GTAACGACAT	TTCAGGCACG	GGCGGCCTGA
1451	TCAAAAAAGG	CGGCAGCCAA	CTGCAACTGC	ACGGCAACAA	CACCTATACG
1501	GGCAAAACCA	TTATCGAAGG	CGGTTTCGTCG	GTGTTGTACG	GCAACAACAA
1551	ATCGGATATG	CGCGTCGAAA	CCAAAGGTGC	GCTGATTTAT	AACGGGGCGG
1601	CATCCGGCGG	CAGCCTGAAC	AGCGACGGCA	TTGTCTATCT	GGCAGATACC
1651	GACCAATCCG	GCGCAACCGA	AACCGTACAC	ATCAAAGGCA	GTCTGCAGCT
1701	GGACGGCAAA	GGTACGCTGT	ACACACGTTT	GGGCAAACTG	CTGAAAGTGG
1751	ACGTACGGC	GATTATCGGC	GGCAAGCTGT	ACATGTCGGC	ACGCGGCAAG
1801	GGGGCAGGCT	ATCTCAACAG	TACCGGACGA	CGTGTTCCTT	TCTTGAGTGC
1851	CGCCAAAATC	GGGCAGGATT	ATTCTTTCTT	CACAAACATC	GAAACCGACG
1901	GCGGCCTGCT	GGCTTCCCTC	GACAGCGTCG	AAAAAACAGC	GGGCAGTGAA
1951	GGCGACACGC	TGTCCTATTA	TGTCCGTCGC	GGCAATGCGG	CACGGACTGC
2001	TTCCGGCAGC	GCACATTCCG	CGCCCGCCCG	TCTGAAACAC	GCCGTAGAAC
2051	AGGGCGGCAG	CAATCTGGAA	AACCTGATGG	TCGAACTGGA	TGCCTCCGAA
2101	TCATCCGCAA	CACCCGAGAC	GGTTGAAACT	GCGGCAGCCG	ACCGCACAGA
2151	TATGCCGGGC	ATCCGCCCCC	ACGGCGCAAC	TTTCCGCGCA	GCGGCAGCCG
2201	TACAGCATGC	GAATGCCGCC	GACGGTGTAC	GCATCTTCAA	CAGTCTCGCC
2251	GCTACCGTCT	ATGCCGACAG	TACCGCCGCC	CATGCCGATA	TGAGGGGACG
2301	CCGCCTGAAA	GCCGTATCGG	ACGGGTGGGA	CCACAACGGC	ACGGGTCTGC
2351	GCGTCATCGC	GCAAAACCAA	CAGGACGGTG	GAACGTGGGA	ACAGGGCGGT
2401	GTTGAAGGCA	AAATGCGCGG	CAGTACCCAA	ACCGTCGGCA	TTGCCCGGAA
2451	AACCGGCGAA	AATACGACAG	CAGCCGCCAC	ACTGGGCATG	GGACGCAGCA
2501	CATGGAGCGA	AAACAGTGCA	AATGCAAAAA	CCGACAGCAT	TAGTCTGTTT
2551	GCAGGCATAC	GGCAGCATGC	GGGCGATATC	GGCTATCTCA	AAGGCCTGTT
2601	CTCCTACGGA	CGCTACAAAA	ACAGCATCAG	CCGCAGCACC	GGTGCAGGAC
2651	AACATGCCGA	AGGCAGCGTC	AACGGCACGC	TGATGCAGCT	GGGCGCACTG
2701	GGCGGTGTCA	ACGTTCCGTT	TGCCGCAACG	GGAGATTTGA	CGGTCTGAAG
2751	CGGTCTGCGC	TACGACCTGC	TCAAACAGGA	TGCATTCGCC	GAAAAAGGCA
2801	GTGCTTTGGG	CTGGAGCGGC	AACAGCCTCA	CTGAAGGCAC	GCTGGTCTGGA
2851	CTCGCGGGTC	TGAAGCTGTC	GCAACCCTTG	AGCGATAAAG	CCGTCTGTGT
2901	TGCAACGGCG	GGCGTGGAA	GCGACCTGAA	CGGACGCGAC	TACACGGTAA
2951	CGGGCGGCTT	TACCGGCGCG	ACTGCAGCAA	CCGGCAAGAC	GGGGGACGCG
3001	AATATGCCGC	ACACCCGTCT	GGTTGCCGGC	CTGGGCGCGG	ATGTCGAATT
3051	CGGCAACGGC	TGGAACGGCT	TGGCACGTTA	CAGCTACGCC	GGTTCCAAAC
3101	AGTACGGCAA	CCACAGCGGA	CGAGTCGGCG	TAGGCTACCG	GTCCTCGAG
3151	GGTGGCGGAG	GCACTGGATC	CGCCACAAAC	GACGACGATG	TTAAAAAAGC
3201	TGCCACTGTG	GCCATTGCTG	CTGCCCTACAA	CAATGGCCAA	GAAATCAACG
3251	GTTTCAAAGC	TGGAGAGACC	ATCTACGACA	TTGATGAAGA	CGGCACAATT
3301	ACCAAAAAAG	ACGCAACTGC	AGCCGATGTT	GAAGCCGACG	ACTTTAAAGG
3351	TCTGGGTCTG	AAAAAAGTCG	TGACTAACCT	GACCAAAACC	GTCAATGAAA
3401	ACAAACAAAA	CGTCGATGCC	AAAGTAAAAG	CTGCAGAATC	TGAAATAGAA
3451	AAGTTAACAA	CCAAGTTAGC	AGACACTGAT	GCCGCTTTAG	CAGATACTGA
3501	TGCCGCTCTG	GATGCAACCA	CCAACGCCTT	GAATAAATTG	GGAGAAAATA
3551	TAACGACATT	TGCTGAAGAG	ACTAAGACAA	ATATCGTAAA	AATTGATGAA

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	3601	AAATTAGAAG	CCGTGGCTGA	TACCGTCGAC	AAGCATGCCG	AAGCATTCAA
	3651	CGATATCGCC	GATTCAATGG	ATGAAACCAA	CACTAAGGCA	GACGAAGCCG
	3701	TCAAAACCGC	CAATGAAGCC	AAACAGACGG	CCGAAGAAAC	CAAACAAAC
5	3751	GTCGATGCCA	AAGTAAAAGC	TGCAGAACT	GCAGCAGGCA	AAGCCGAAGC
	3801	TGCCGCTGGC	ACAGCTAATA	CTGCAGCCGA	CAAGGCCGAA	GCTGTGCGTG
	3851	CAAAAGTTAC	CGACATCAAA	GCTGATATCG	CTACGAACAA	AGATAATATT
	3901	GCTAAAAAAG	CAAAACAGTGC	CGACGTGTAC	ACCAGAGAAG	AGTCTGACAG
	3951	CAAATTTGTC	AGAATTGATG	GTCTGAACGC	TACTACCGAA	AAATTGGACA
10	4001	CACGCTTGGC	TTCTGCTGAA	AAATCCATTG	CCGATCACGA	TACTCGCCTG
	4051	AACGGTTTGG	ATAAACAGT	GTCAGACCTG	CGCAAAGAAA	CCCGCCAAGG
	4101	CCTTGACAGAA	CAAGCCGCGC	TCTCCGGTCT	GTTCCAACCT	TACAACGTGG
	4151	GTCTCGAGCA	CCACCACCAC	CACCACTGA		
	1	MTSAPDFNAG	GTGIGSNSRA	TTAKSAAVS	AGIKNEMCKD	RSMLCAGRDD
15	51	VAVTDRDAKI	NAPPPNLHTG	DFPNPNDAYK	NLINLKPAIE	AGYTGRGVEV
	101	GIVDTGESVG	SISFPELYGR	KEHGYNENYK	NYTAYMRKEA	PEDGGGKDIE
	151	ASFDDEAVIE	TEAKPTDIRH	VKEIGHIDL	SHIIGGRSVD	GRPAGGIAPD
	201	ATLHIMNTND	ETKNEMMVAA	IRNAWVKLGE	RGVRIVNNSF	GTTSRAGTAD
	251	LFQIANSEEQ	YRQALLDYS	GDKTDEGIRL	MQQSDYGNLS	YHIRKNMFL
20	301	IFSTGNDAQA	QPNTYALLPF	YEKDAQKGII	TVAGVDRSGE	KFKREMYGEP
	351	GTEPLEYGSN	HCGITAMWCL	SAPYEASVRF	TRTNPIQIAG	TSFSAPIVTG
	401	TAALLLQKYP	WMSNDNLRTT	LLTTAQDIGA	VGVDKFGWG	LLDAGKAMNG
	451	PASFPFGDFT	ADTKGTS DIA	YSFRNDISGT	GGLIKKGGSQ	LQLHGNNTYT
25	501	GKTIIEGGS	VLYGNKSDM	RVETKGALII	NGAASGGS LN	SDGIVYLADT
	551	DQSGANETVH	IKGSLQLDGK	GTLYTRLGKL	LKVDGTAIIG	GKLYMSARGK
	601	GAGYLNSTGR	RVPFLSAAKI	GQDYSFFTNI	ETDGGLLASL	DSVEKTAGSE
	651	GDTLSYYVRR	GNAARTASAA	AHSAPAGLKH	AVEQGGSNLE	NLMVELDASE
	701	SSATPETVET	AAADRTDMPG	IRPYGATFRA	AAAVQHANA	DGVIRIFNSLA
30	751	ATVYADSTAA	HADMQRRLK	AVSDGLDHNG	TGLRVIAQTQ	QDGGTWEQGG
	801	VEGKMRGSTQ	TVGIAAKTGE	NTTAAATLGM	GRSTWSENSA	NAKTDSISLF
	851	AGIRHDAGDI	GYLKG LFSYG	RYKNSISRST	GADEHAEGSV	NGTLMQLGAL
	901	GGVNVPPFAAT	GDLTVEGGLR	YDLLKQDAFA	EKGSALGWSG	NSLTEGTLVG
	951	LAGLKL SQPL	SDKAVLFATA	GVERDLNGRD	YTVTG GFTGA	TAATGKTGAR
35	1001	NMPHTRLVAG	LGADVEFGNG	WNGLARYSYA	GSKQYGNHSG	RVGVGYRFL
	1051	GGGGTGSATN	DDDVKKAATV	AIAAAYNNGQ	EINGFKAGET	IYDIDEDGTI
	1101	TKKDATAADV	EADDFKGLGL	KKVVTNLTKT	VNENKQNVDA	KVKAASEEIE
	1151	KLTTKLADTD	AALADTDAAL	DATTNALNKL	GENITTFAE	TKTNIVKIDE
	1201	KLEAVADTV	KhAEAFNDIA	DSLDETNTKA	DEAVKTANEA	KQTAETKQN
40	1251	VDAKVKAET	AAGKAEAAA	TANTAADKAE	AVAAKVTDIK	ADIATNKDNI
	1301	AKKANSADV	TREESDSKFV	RIDGLNATTE	KLDTRLASAE	KSIADHDTRL
	1351	NGLDKTVSDL	RKETRQGLAE	QAALSGLFQP	YNVGLEHHHH	HH*

*ΔG741 and hybrids*

Bactericidal titres generated in response to ΔG741 (His-fusion) were measured against various strains, including the homologous 2996 strain:

	2996	MC58	NGH38	F6124	BZ133
<b>ΔG741</b>	512	131072	>2048	16384	>2048

45 As can be seen, the ΔG741-induced anti-bactericidal titre is particularly high against heterologous strain MC58.

ΔG741 was also fused directly in-frame upstream of proteins 961, 961c, 983 and ORF46.1:

	<b>ΔG741-961</b>					
50	1	ATGGTCGCCG	CCGACATCGG	TGCGGGGCTT	GCCGATGCAC	TAACCGCACC
	51	GCTCGACCAT	AAAGACAAAG	GTTTGCAGTC	TTTGACGCTG	GATCAGTCCG
	101	TCAGGAAAAA	CGAGAACTG	AAGCTGGCGG	CACAAGGTGC	GGAAAAACT
	151	TATGGAAACG	GTGACAGCCT	CAATACGGGC	AAATTGAAGA	ACGACAAGGT
	201	CAGCCGTTTC	GACTTTATCC	GCCAAATCGA	AGTGGACGGG	CAGCTCATT

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251 CCTTGGAGAG TGGAGAGTTC CAAGTATACA AACAAAGCCA TTCCGCCTTA  
 301 ACCGCCTTTC AGACCGAGCA AATACAAGAT TCGGAGCATT CCGGGAAGAT  
 351 GGTTGCGAAA CGCCAGTTCA GAATCGGCGA CATAGCGGGC GAACATACAT  
 401 CTTTTGACAA GCTTCCCGAA GGCGGCAGGG CGACATATCG CCGGACGGCG  
 5 451 TTCGGTTCAG ACGATGCCGG CGGAAACTG ACCTACACCA TAGATTTCCG  
 501 CGCCAAGCAG GGAAACGGCA AAATCGAACA TTTGAAATCG CCAGAACTCA  
 551 ATGTCGACCT GGCCGCCGCC GATATCAAGC CGGATGGAAA ACGCCATGCC  
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 10 701 TGAAAACCGT AAACGGCATA CGCCATATCG GCCTTGCCGC CAAGCAACTC  
 751 GAGGGTGGCG GAGGCACTGG ATCCGCCACA AACGACGACG ATGTTAAAAA  
 801 AGCTGCCACT GTGGCCATTG CTGCTGCCTA CAACAATGGC CAAGAAATCA  
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 15 951 AGGTCTGGGT CTGAAAAAAG TCGTGACTAA CCTGACCAA ACCGTCAATG  
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 20 1201 GAAAAATTAG AAGCCGTGGC TGATACCGTC GACAAGCATG CCGAAGCATT  
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 1401 AGCTGCCGCT GGCACAGCTA ATACTGCAGC CGACAAGGCC GAAGCTGTCTG  
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 1 MVAADIGAGL ADALTAPLDH KDKGLQSLTL DQSVRKNEKL KLAAQGAIEKT  
 51 YNGDSLNTG KLKNDKVSFR DFIRQIEVDG QLITLESGEF QVYKQSHSAL  
 101 TAFQTEQIQD SEHSGKMKVAK RQFRIGDIAG EHTSFDKLPE GGRATYRGTA  
 151 FGSDDAGGKL TYTIDFAAKQ GNGKIEHLKS PELNVDLAAA DIKPDGKRHA  
 40 201 VISGSVLYNQ AEKGSYSLSI FGGKAQEVAG SAEVKTVNGI RHIGLAAKQL  
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 351 EKLTTKLADT DAALADTDAA LDATTNALNK LGENITTFAE ETKTNIVKID  
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 45 451 NVDAKVKAEE TAAGKAEAAA GTANTAADKA EAVAAKVTDI KADIATNKDN  
 501 IAKKANSADV YTREESDSKF VRIDGLNATT EKLDTRLASA EKSIADHDTR  
 551 LNGLDKTVSD LRKETRQGLA EQAALSGLFQ PYNVGRFNV AAVGGYKSES  
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1 ATGGTCGCCG CCGACATCGG TGCGGGGCTT GCCGATGCAC TAACCGCACC  
 51 GCTCGACCAT AAAGACAAAG GTTTGCAGTC TTTGACGCTG GATCAGTCCG  
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 201 CAGCCGTTTC GACTTTATCC GCCAAATCGA AGTGGACGGG CAGCTCATTA  
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 65 651 CCTCGGTATC TTTGGCGGAA AAGCCCAGGA AGTTGCCGGC AGCGCGGAAG  
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5	801	AGCTGCCACT	GTGGCCATTG	CTGCTGCCTA	CAACAATGGC	CAAGAAATCA
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	951	AGGTCTGGGT	CTGAAAAAAG	TCGTGACTAA	CCTGACCAAA	ACCGTCAATG
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	1351	AACGTTCGATG	CCAAAGTAAA	AGCTGCAGAA	ACTGCAGCAG	GCAAAGCCGA
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	1451	CTGCAAAAGT	TACCGACATC	AAAGCTGATA	TCGCTACGAA	CAAAAGATAAT
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	1601	ACACACGCTT	GGCTTCTGCT	GAAAAATCCA	TTGCCGATCA	CGATACTCGC
	1651	CTGAACGGTT	TGGATAAAAC	AGTGTCAGAC	CTGCGCAAAG	AAACCCGCCA
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	1751	TGGGTCTCGA	GCACCACCAC	CACCACCACT	GA	
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25	101	TAFQTEQIQD	SEHSGKMVAK	RQFRIGDIAG	EHTSFDKLPE	GGRATYRGTA
	151	FGSDDAGGKL	TYTIDFAAKQ	GNGKIEHLKS	PELNVDLAAA	DIKPDGKRHA
	201	VISGSVLYNQ	AEKGSYSLGI	FGGKAQEVAG	SAEVKTVNGI	RHIGLAARQL
	251	EGGGGTGSAT	NDDDVKKAAAT	VAIAAAYMNG	QEINGFKAGE	TIYDIDEDGT
	301	ITKKDATAAD	VEADDFKGLG	LKKVVTNLTK	TVNENKQNV	AKVKAASEI
30	351	EKLTTKLADT	DAALADTDAA	LDATTNALNK	LGENTTTFAE	ETKTNIVKID
	401	EKLEAVADTV	DKHAEAFNDI	ADSLDETNTK	ADEAVKTANE	AKQTAEBTKQ
	451	NVDAKVKAEE	TAAGKAEAAA	GTANTAADKA	EAVAAKVTDI	KADIATNKDN
	501	IACKANSADV	YTREESDSKF	VRIDGLNATT	EKLDTRLASA	EKSIADHDTR
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35		<u>AG741-983</u>				
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	51	GCTCGACCAT	AAAGACAAAG	GTTTGACGTC	TTTGACGCTG	GATCAGTCCG
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	201	CAGCCGTTTC	GACTTTATCC	GCCAAATCGA	AGTGGACGGG	CAGCTCATTA
	251	CCTTGAGAG	TGGAGAGTTC	CAAGTATACA	AACAAAGCCA	TTCCGGCTTA
	301	ACCGCCTTTC	AGACCGAGCA	AATACAAGAT	TCGGAGCATT	CCGGGAAGAT
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45	401	CTTTTGACAA	GCTTCCCGAA	GGCGGCAGGG	CGACATATCG	CGGGACGGCG
	451	TTCCGGTTCAG	ACGATGCCGG	CGGAAAACTG	ACCTACACCA	TAGATTTTCGC
	501	CGCCAAGCAG	GGAAACGGCA	AAATCGAACA	TTTGAAATCG	CCAGAACTCA
	551	ATGTCGACCT	GGCCGCGGCC	GATATCAAGC	CGGATGGAAA	ACGCCATGCC
	601	GTCATCAGCG	GTTCCGTCCT	TTACAACCAA	GCCGAGAAAG	GCAAGTTACTC
50	651	CCTCGGTATC	TTTGGCGGAA	AAGCCCAGGA	AGTTGCCGGC	AGCGCGGAAG
	701	TGAAAACCGT	AAACGGCATA	CGCCATATCG	GCCTTGCCGC	CAAGCAACTC
	751	GAGGGATCCG	GCGGAGGCGG	CACTTCTGCG	CCCGACTTCA	ATGCAGGCGG
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	851	TATCTTACGC	CGGTATCAAG	AACGAAATGT	GCAAAGACAG	AAGCATGCTC
55	901	TGTGCCGGTC	GGGATGACGT	TGCGGTTACA	GACAGGGATG	CCAAAATCAA
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	1001	CATACAAGAA	TTTGATCAAC	CTCAAACCTG	CAATTGAAGC	AGGCTATACA
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	1101	CATATCCTTT	CCCGAAGCTGT	ATGGCAGAAA	AGAACACGGC	TATAACGAAA
60	1151	ATTACAAAAA	CTATACGGCG	TATATGCGGA	AGGAAGCGCC	TGAAGACGGA
	1201	GGCGGTAAAG	ACATTGAAGC	TTCTTTTCGAC	GATGAGGCCG	TTATAGAGAC
	1251	TGAAGCAAAG	CCGACGGATA	TCCGCCACGT	AAAAGAAATC	GGACACATCG
	1301	ATTTGGTCTC	CCATATTATT	GGCGGGCGTT	CCGTGGACGG	CAGACCTGCA
	1351	GGCGGTATTG	CGCCCGATGC	GACGCTACAC	ATAATGAATA	CGAATGATGA
65	1401	AACCAAGAAC	GAAATGATGG	TTGCAGCCAT	CCGCAATGCA	TGGGTCAAGC
	1451	TGGGCGAACG	TGGCGTGC	ATCGTCAATA	ACAGTTTGG	AACAACATCG
	1501	AGGGCAGGCA	CTGCCGACCT	TTTCCAAATA	GCCAATTCGG	AGGAGCAGTA

5	1551	CCGCCAAGCG	TTGCTCGACT	ATTCCGGCGG	TGATAAAACA	GACGAGGGTA	
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	1651	AATAAAAACA	TGCTTTTTCAT	CTTTTCGACA	GGCAATGACG	CACAAGCTCA	
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	1751	GCATTATCAC	AGTCGCAGGC	GTAGACCCGA	GTGGAGAAAA	GTTCAAACGG	
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	1951	GCACCCATCG	TAACCGGCAC	GGCGGCTCTG	CTGCTGCAGA	AATACCCGTG	
	2001	GATGAGCAAC	GACAACCTGC	GTACCACGTT	GCTGACGACG	GCTCAGGACA	
10	2051	TCCGTGTCAGT	CGGCGTGGAC	AGCAAGTTTCG	GCTGGGGACT	GCTGGATGCG	
	2101	GGTAAGGCCA	TGAACGGACC	CGCGTCCTTT	CCGTTCCGGC	ACTTTACCG	
	2151	CGATACGAAA	TGTACATCCG	ATATTGCCTA	CTCCTTCCGT	AACGACATTT	
	2201	CAGGCACGGG	CGGCCTGATC	AAAAAAGGCG	GCAGCCAACT	GCAACTGCAC	
	2251	GGCAACAACA	CCTATACGGG	CAAAACCATT	ATCGAAGGCG	GTTCGCTGGT	
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	2351	TGATTTATAA	CGGGGCGGCA	TCCGGCGGCA	GCCTGAACAG	CGACGGCATT	
	2401	GTATTCTTGG	CAGATACCGA	CCAATCCGGC	GCAAACGAAA	CCGTACACAT	
	2451	CAAAGGCAGT	CTGCAGCTGG	ACGGCAAAGG	TACGCTGTAC	ACACGTTTGG	
	2501	GCAAACCTGCT	GAAAGTGGAC	GGTACGGCGA	TTATCGGCGG	CAAGCTGTAC	
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	2701	AAAACAGCGG	GCAGTGAAGG	CGACACGCTG	TCCTATTATG	TCCGTGCGGG	
	2751	CAATGCGGCA	CGGACTGCTT	CGGCAGCGGC	ACATTCCGGC	CCCGCCGGTC	
	2801	TGAAACACGC	CGTAGAACAG	GGCGGCAGCA	ATCTGGAATA	CCTGATGGTC	
	2851	GAAGTGGATG	CCTCCGAATC	ATCCGCAACA	CCCGAGACGG	TTGAAACTGC	
	2901	GGCAGCCGAC	CGCACAGATA	TGCCGGGCAT	CCGCCCCTAC	GGCGCAACTT	
	2951	TCCCGCGCAG	GGCAGCCGTA	CAGCATGCGA	ATGCCGCCGA	CGGTGTACGC	
	3001	ATCTTCAACA	GTCTCGCCGC	TACCGTCTAT	GCCGACAGTA	CCGCCGCCCA	
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	3701	GACGCGACTA	CACGGTAACG	GGCGGCTTTA	CCGGCGCGAC	TGCAGCAACC	
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	50	1	MVAADIGAGL	ADALTAPLDH	KDKGLQSLTL	DQSVRKNEKL	KLAAQGAERT
	51	YNGDSLNTG	KLKNDKVSFP	DFIRQIEVDG	QLITLESSEF	QVYKQSHSAL	
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201	VISGSVLYNQ	AEKGSYSLGI	FGGKAQEVAG	SAEVKTVNGI	RHIGLAAKQL		
251	EGSGGGGTS	PDFNAGGTGI	GSNSRATTAK	SAAVSYAGIK	NEMCKDRSML		
301	CAGRDDVAVT	DRDAKINAPP	PNLHTGDFPN	PNDAYKNLIN	LKPAIEAGYT		
351	GRGVEVGIVD	TGESVGSISF	PELYGRKEHG	YNENYKNYTA	YMRKEAPEDG		
401	GGKDIEASFD	DEAVIETKAK	PTDIRHVKEI	GHIDLVSII	GGRSVDGRPA		
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501	RAGTADLFQI	ANSEEQYRQA	LLDYSGGDKT	DEGIRLMQOS	DYGNLSYHIR		
551	NKNMLFIFST	GNDQAQPNPT	YALLPFYEKD	AQKGIITVAG	VDRSGEKFR		
601	EMYGEPGTEP	LEYGSNHCGI	TAMWCLSAPY	EASVRFTRTN	PIQIAGTSFS		
651	APIVTGTAAL	LLQKYPWMSN	DNLRTTLLTT	AQDIGAVGVD	SKFGWGLLDA		
701	GKAMNGPASF	PFGDFTADTK	GTSDIAYSFR	NDISGTGGLI	KKGGSQQLQH		
751	GNNTYTGKTI	IEGGSLLVLYG	NNKSMDMRVET	KGALYNGAA	SGGSLNSDGI		
801	VYLADTDQSG	ANETVHIKGS	LQLDGKGTLY	TRLGKLLKVD	GTAIIGGKLY		
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1001	IFNSLAATVY	ADSTAAHADM	QGRRLKAVSD	GLDHNGTGLR	VIAQTQODGG
1051	TWEQGGVEGK	MRGSTQTVGI	AAKTGENTTA	AATLGMGRST	WSENSANAKT
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1151	MQLGALGGVN	VPFAATGDLT	VEGGLRYDLL	KQDAFAEKG	ALGWSGNSLT
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1301	GYRFLEHHHH	HH*			

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951	CATTAAAGGA	AATATCGGCT	ACATTGTCCG	CTTTTCCGAT	CACGGGCACG
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1551	ACTCAAAGAC	TATGCCGCGC	CAGCCATCCG	CGATTGGGCA	GTCCAAAACC
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1751	CGAAAGGGAA	ATCCGCCGTC	AGCGACAATT	TTGCCGATGC	GGCATAACGC
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1851	CGGTACGGC	AAAGAAAAACA	TCACCTCCTC	AACCGTGCCG	CCGTCAAACG
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1951	TTTGACGGTA	AAGGGTTTCC	GAATTTTGAG	AAGCACGTGA	AATATGATAC
2001	GCTCGAGCAC	CACCACCACC	ACCACTGA		

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1	MVAADIGAGL	ADALTAPLDH	KDKGLQSLTL	DQSVRKNEKL	KLAAQGAECT
51	YNGDSLNTG	KLKNDKVSF	DFIRQIEVDG	QLITLESGEF	QVYKQSHSAL
101	TAFQTEQIQD	SEHSGKMVAR	RQFRIGDIAG	EHTSFDKLPE	GGRATYRGTA
151	FGSDDAGGKL	TYTIDFAAKQ	GNGKIEHLKS	PELVNDLAAA	DIKPDGKRHA
201	VISGSVLYNQ	AEKGSYSLGI	FGGKAQEVAG	SAEVKTVNGI	RHIGLAAKQL
251	DGGGGTGSSD	LANDSFIRQV	LDRQHFEFDG	KYHLFGSRGE	LAERSGHICL
301	GKIQSHQLGN	LMIQQAIAKG	NIGYIVRFS	HGHEVHSPFD	NHASHSDSDE
351	AGSPVDGFS	YRIHWGDYEH	HPADGYDGPQ	GGGYPAKGA	RDIYSYDIK
401	VAQNIRLNL	DNRSTGQRLA	DRFHNGSML	TQGVGDGFKR	ATRYSPELDR
451	SGNAAEAFNG	TADIVKNIIG	AAGEIVGAGD	AVQGISSEGSN	IAMVHGLGLL
501	STENKMARIN	DLADMAQLKD	YAAAAIRDWA	VQNPNAAQGI	EAVSNIFMAA
551	IPIKGIGAVR	GRYGLGGITA	HPIKRSQMG	IALPKGKSAV	SDNFADAAYA
601	KYPSPYHSRN	IRSNLEQRYG	KENITSSTVP	PSNGKNVKLA	DQRHPKTGVP
651	FDGKGFPNFE	KHVKYDTLEH	HHHHH*		

**Example 16 – C-terminal fusions ('hybrids') with 287/ $\Delta$ G287**

According to the invention, hybrids of two proteins A & B may be either NH<sub>2</sub>-A-B-COOH or NH<sub>2</sub>-B-A-COOH. The effect of this difference was investigated using protein 287 either C-terminal (in '287-His' form) or N-terminal (in  $\Delta$ G287 form – sequences shown above) to

5 919, 953 and ORF46.1. A panel of strains was used, including homologous strain 2996. FCA was used as adjuvant:

	<b>287 &amp; 919</b>		<b>287 &amp; 953</b>		<b>287 &amp; ORF46.1</b>	
Strain	$\Delta$ G287-919	919-287	$\Delta$ G287-953	953-287	$\Delta$ G287-46.1	46.1-287
<b>2996</b>	128000	16000	65536	8192	16384	8192
<b>BZ232</b>	256	128	128	<4	<4	<4
<b>1000</b>	2048	<4	<4	<4	<4	<4
<b>MC58</b>	8192	1024	16384	1024	512	128
<b>NGH38</b>	32000	2048	>2048	4096	16384	4096
<b>394/98</b>	4096	32	256	128	128	16
<b>MenA (F6124)</b>	32000	2048	>2048	32	8192	1024
<b>MenC (BZ133)</b>	64000	>8192	>8192	<16	8192	2048

Better bactericidal titres are generally seen with 287 at the N-terminus (in the  $\Delta$ G form)

When fused to protein 961 [NH<sub>2</sub>- $\Delta$ G287-961-COOH – sequence shown above], the resulting protein is insoluble and must be denatured and renatured for purification. Following

10 renaturation, around 50% of the protein was found to remain insoluble. The soluble and insoluble proteins were compared, and much better bactericidal titres were obtained with the soluble protein (FCA as adjuvant):

	<b>2996</b>	<b>BZ232</b>	<b>MC58</b>	<b>NGH38</b>	<b>F6124</b>	<b>BZ133</b>
<b>Soluble</b>	65536	128	4096	>2048	>2048	4096
<b>Insoluble</b>	8192	<4	<4	16	n.d.	n.d.

Titres with the insoluble form were, however, improved by using alum adjuvant instead:

<b>Insoluble</b>	32768	128	4096	>2048	>2048	2048
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**Example 17 – N-terminal fusions ('hybrids') to 287**

Expression of protein 287 as full-length with a C-terminal His-tag, or without its leader peptide but with a C-terminal His-tag, gives fairly low expression levels. Better expression is achieved using a N-terminal GST-fusion.

As an alternative to using GST as an N-terminal fusion partner, 287 was placed at the C-terminus of protein 919 ('919-287'), of protein 953 ('953-287'), and of proteins ORF46.1 ('ORF46.1-287'). In both cases, the leader peptides were deleted, and the hybrids were direct in-frame fusions.

- 5 To generate the 953-287 hybrid, the leader peptides of the two proteins were omitted by designing the forward primer downstream from the leader of each sequence; the stop codon sequence was omitted in the 953 reverse primer but included in the 287 reverse primer. For the 953 gene, the 5' and the 3' primers used for amplification included a *NdeI* and a *BamHI* restriction sites respectively, whereas for the amplification of the 287 gene the 5' and the 3' primers included a *BamHI* and a *XhoI* restriction sites respectively. In this way a sequential directional cloning of the two genes in pET21b+, using *NdeI-BamHI* (to clone the first gene) and subsequently *BamHI-XhoI* (to clone the second gene) could be achieved.

- 15 The 919-287 hybrid was obtained by cloning the sequence coding for the mature portion of 287 into the *XhoI* site at the 3'-end of the 919-His clone in pET21b+. The primers used for amplification of the 287 gene were designed for introducing a *SalI* restriction site at the 5'- and a *XhoI* site at the 3'- of the PCR fragment. Since the cohesive ends produced by the *SalI* and *XhoI* restriction enzymes are compatible, the 287 PCR product digested with *SalI-XhoI* could be inserted in the pET21b-919 clone cleaved with *XhoI*.

The ORF46.1-287 hybrid was obtained similarly.

- 20 The bactericidal efficacy (homologous strain) of antibodies raised against the hybrid proteins was compared with antibodies raised against simple mixtures of the component antigens:

	Mixture with 287	Hybrid with 287
<b>919</b>	32000	16000
<b>953</b>	8192	8192
<b>ORF46.1</b>	128	8192

Data for bactericidal activity against heterologous MenB strains and against serotypes A and C were also obtained for 919-287 and 953-287:

	919		953		ORF46.1	
Strain	Mixture	Hybrid	Mixture	Hybrid	Mixture	Hybrid
MC58	512	1024	512	1024	-	1024
NGH38	1024	2048	2048	4096	-	4096
BZ232	512	128	1024	16	-	-
MenA (F6124)	512	2048	2048	32	-	1024
MenC (C11)	>2048	n.d.	>2048	n.d.	-	n.d.
MenC (BZ133)	>4096	>8192	>4096	<16	-	2048

Hybrids of ORF46.1 and 919 were also constructed. Best results (four-fold higher titre) were achieved with 919 at the N-terminus.

Hybrids 919-519His, ORF97-225His and 225-ORF97His were also tested. These gave moderate ELISA titres and bactericidal antibody responses.

#### 5 Example 18 – the leader peptide from ORF4

As shown above, the leader peptide of ORF4 can be fused to the mature sequence of other proteins (e.g. proteins 287 and 919). It is able to direct lipidation in *E.coli*.

#### Example 19 – domains in 564

The protein '564' is very large (2073aa), and it is difficult to clone and express it in complete form. To facilitate expression, the protein has been divided into four domains, as shown in figure 8 (according to the MC58 sequence):

Domain	A	B	C	D
Amino Acids	79-360	361-731	732-2044	2045-2073

These domains show the following homologies:

#### • Domain A shows homology to other bacterial toxins:

- 15 gb|AAG03431.1|AE004443\_9probable hemagglutinin [*Pseudomonas aeruginosa*] (38%)  
 gb|AAC31981.1|(139897) HecA [*Pectobacterium chrysanthemi*] (45%)  
 emb|CAA36409.1|(X52156) filamentous hemagglutinin [*Bordetella pertussis*] (31%)  
 gb|AAC79757.1|(AF057695) large supernatant protein1 [*Haemophilus ducreyi*] (26%)  
 gb|AAA25657.1|(M30186) Hpma precursor [*Proteus mirabilis*] (29%)

#### 20 • Domain B shows no homology, and is specific to 564.

#### • Domain C shows homology to:

- 25 gb|AAF84995.1|AE004032 HA-like secreted protein [*Xylella fastidiosa*] (33%)  
 gb|AAG05850.1|AE004673 hypothetical protein [*Pseudomonas aeruginosa*] (27%)  
 gb|AAF68414.1|AF237928 putative FHA [*Pasteurella multocida*] (23%)  
 gb|AAC79757.1|(AF057695) large supernatant protein1 [*Haemophilus ducreyi*] (23%)  
 pir||S21010 FHA B precursor [*Bordetella pertussis*] (20%)

- Domain D shows homology to other bacterial toxins:

gb|AAF84995.1|AE004032\_14 HA-like secreted protein [*Xylella fastidiosa*] (29%)

Using the MC58 strain sequence, good intracellular expression of 564ab was obtained in the form of GST-fusions (no purification) and his-tagged protein; this domain-pair was also expressed as a lipoprotein, which showed moderate expression in the outer membrane/supernatant fraction.

The b domain showed moderate intracellular expression when expressed as a his-tagged product (no purification), and good expression as a GST-fusion.

The c domain showed good intracellular expression as a GST-fusion, but was insoluble. The d domain showed moderate intracellular expression as a his-tagged product (no purification). The cd protein domain-pair showed moderate intracellular expression (no purification) as a GST-fusion.

Good bactericidal assay titres were observed using the c domain and the bc pair.

#### Example 20 – the 919 leader peptide

The 20mer leader peptide from 919 is discussed in example 1 above:

MKKYLFRAAL YGIAAAILAA

As shown in example 1, deletion of this leader improves heterologous expression, as does substitution with the ORF4 leader peptide. The influence of the 919 leader on expression was investigated by fusing the coding sequence to the *PhoC* reporter gene from *Morganella morganii* [Thaller *et al.* (1994) *Microbiology* 140:1341-1350]. The construct was cloned in the pET21-b plasmid between the *NdeI* and *XhoI* sites (Figure 9):

1	MKKYLFRAAL	YGIAAAILAA	AIPAGNDATT	KPDLYYLKNE	QAIDSLKLLP
51	PPPEVGSIQF	LNDQAMYKEG	RMLRNTERGK	QAQADADLAA	GGVATAFSGA
101	FGYPITEKDS	PELYKLLTNM	IEDAGDLATR	SAKEHYMRIR	PFAFYGTETC
151	NTKDQKKLST	NGSYPSGHTS	IGWATALVLA	EVNPNANQDAI	LERGYQLGQS
201	RVICGYHWQS	DVDAARIVGS	AAVATLHSDP	AFQAQLAKAK	QEFAQKSQK*

The level of expression of *PhoC* from this plasmid is >200-fold lower than that found for the same construct but containing the native *PhoC* signal peptide. The same result was obtained even after substitution of the T7 promoter with the *E.coli* Plac promoter. This means that the influence of the 919 leader sequence on expression does not depend on the promoter used.

In order to investigate if the results observed were due to some peculiarity of the 919 signal peptide nucleotide sequence (secondary structure formation, sensitivity to RNAases, *etc.*) or

to protein instability induced by the presence of this signal peptide, a number of mutants were generated. The approach used was a substitution of nucleotides of the 919 signal peptide sequence by cloning synthetic linkers containing degenerate codons. In this way, mutants were obtained with nucleotide and/or amino acid substitutions.

- 5 Two different linkers were used, designed to produce mutations in two different regions of the 919 signal peptide sequence, in the first 19 base pairs (L1) and between bases 20-36 (S1).

10 **L1:** 5' T ATG AAa/g TAc/t c/tTN TtT/c a/cGC GCC GCC CTG TAC GGC ATC GCC GCC  
GCC ATC CTC GCC GCC GCG ATC CC 3'  
**S1:** 5' T ATG AAA AAA TAC CTA TTC CGa/g GCN GCN c/tTa/g TAc/t GGc/g ATC GCC  
GCC GCC ATC CTC GCC GCC GCG ATC CC 3'

The alignment of some of the mutants obtained is given below.

**L1 mutants:**

15 9L1-a ATGAAGAAGTACCTTTTCAGCGCCGCC~  
9L1-e ATGAAAAAATACTTTTTCGCGCCGCC~  
9L1-d ATGAAAAAATACTTTTTCGCGCCGCC~  
9L1-f ATGAAAAAATATCTTTAGCGCGCCCTGTACGGCATCGCCCGCCATCCTCGCCGCC  
919sp ATGAAAAAATACCTATTTCGCGCCGCCCTGTACGGCATCGCCCGCCATCCTCGCCGCC

20 9L1a MKKYLFSAA~  
9L1e MKKYFFRAA~  
9L1d MKKYFFRAA~  
9L1f MKKYLFSAAALYGIAAAILAA  
919sp MKKYLFRAAALYGIAAAILAA (i.e. native signal peptide)

25 **S1 mutants:**

9S1-e ATGAAAAAATACCTATTC.....ATCGCCGCGCCATCCTCGCCGCC  
9S1-c ATGAAAAAATACCTATTCGAGCTGCCCAATACGGCATCGCCGCGCCATCCTCGCCGCC  
30 9S1-b ATGAAAAAATACCTATTCGGGCGCCCAATACGGCATCGCCGCGCCATCCTCGCCGCC  
9S1-i ATGAAAAAATACCTATTCGGGCGGCTTTGTACGGGATCGCCGCGCCATCCTCGCCGCC  
919sp ATGAAAAAATACCTATTCGCGCGCCGCCCTGTACGGCATCGCCGCGCCATCCTCGCCGCC

35 9S1e MKKYL.....IAAAILAA  
9S1c MKKYLFRAAQYGIAAAILAA  
9S1b MKKYLFRAAQYGIAAAILAA  
9S1i MKKYLFRAAALYGIAAAILAA  
919sp MKKYLFRAAALYGIAAAILAA

- 40 As shown in the sequences alignments, most of the mutants analysed contain in-frame deletions which were unexpectedly produced by the host cells.

Selection of the mutants was performed by transforming *E. coli* BL21(DE3) cells with DNA prepared from a mixture of L1 and S1 mutated clones. Single transformants were screened for high PhoC activity by streaking them onto LB plates containing 100 µg/ml ampicillin,  
45 50µg/ml methyl green, 1 mg/ml PDP (phenolphthaleindiphosphate). On this medium PhoC-producing cells become green (Figure 10).

A quantitative analysis of PhoC produced by these mutants was carried out in liquid medium using pNPP as a substrate for PhoC activity. The specific activities measured in cell extracts and supernatants of mutants grown in liquid medium for 0, 30, 90, 180 min. were:

### CELL EXTRACTS

	0	30	90	180
control	0,00	0,00	0,00	0,00
9phoC	1,11	1,11	3,33	4,44
9S1e	102,12	111,00	149,85	172,05
9L1a	206,46	111,00	94,35	83,25
9L1d	5,11	4,77	4,00	3,11
9L1f	27,75	94,35	82,14	36,63
9S1b	156,51	111,00	72,15	28,86
9S1c	72,15	33,30	21,09	14,43
9S1i	156,51	83,25	55,50	26,64
phoCwt	194,25	180,93	149,85	142,08

5

### SUPERNATANTS

	0	30	90	180
control	0,00	0,00	0,00	0,00
9phoC	0,33	0,00	0,00	0,00
9S1e	0,11	0,22	0,44	0,89
9L1a	4,88	5,99	5,99	7,22
9L1d	0,11	0,11	0,11	0,11
9L1f	0,11	0,22	0,11	0,11
9S1b	1,44	1,44	1,44	1,67
9S1c	0,44	0,78	0,56	0,67
9S1i	0,22	0,44	0,22	0,78
phoCwt	34,41	43,29	87,69	177,60

Some of the mutants produce high amounts of PhoC and in particular, mutant 9L1a can secrete PhoC in the culture medium. This is noteworthy since the signal peptide sequence of this mutant is only 9 amino acids long. This is the shortest signal peptide described to date.

10

### *Example 21 – C-terminal deletions of Maf-related proteins*

MafB-related proteins include 730, ORF46 and ORF29.

The 730 protein from MC58 has the following sequence:

15

20

```

1  VKPLRRLTNL LAACAVAAAA LIQPALAADL AQDPFITDNA QRQHYEPGGK
51  YHLFGDPRGS VSDRTGKINV IQDYTHQMGN LLIQQANING TIGYHTRFSG
101 HGHEEHAPFD NHAADSASEE KGNVDEGFTV YRLNWEGHEH HPADAYDGPK
151 GGNYPKPTGA RDEYTYHVNG TARSIKLNPT DTRSIRQRIS DNYSNLGSNF
201 SDRADEANRK MFEHNAKLDR WGNSMEFING VAAGALNPFI SAGEALGIGD
251 ILYGTRYAID KAAMRNIAPI PAEGKFAVIG GLGSVAGFEK NTREAVDRWI
301 QENPNAAETV EAVFNVAAAA KVAKLAKAAK PGKAAVSGDF ADSYKKKLAL

```

351 SDSARQLYQN AKYREALDIH YEDLIRRKTD GSSKFINGRE IDAVTNDALI  
 401 QAKRTISAID KPNFLNQKN RKQIKATIEA ANQQGKRAEF WFKYGVHSQV  
 451 KSYIESKGGI VRTGLGD\*

- 5 The leader peptide is underlined.

730 shows similar features to ORF46 (see example 8 above):

- as for Orf46, the conservation of the 730 sequence among MenB, MenA and gonococcus is high (>80%) only for the N-terminal portion. The C-terminus, from ~340, is highly divergent.
- 10 - its predicted secondary structure contains a hydrophobic segment spanning the central region of the molecule (aa. 227-247).
- expression of the full-length gene in *E. coli* gives very low yields of protein. Expression from tagged or untagged constructs where the signal peptide sequence has been omitted has a toxic effect on the host cells. In other words, the presence of the full-length mature protein in the cytoplasm is highly toxic for the host cell while its translocation to the periplasm (mediated by the signal peptide) has no detectable effect on cell viability. This "intracellular toxicity" of 730 is particularly high since clones for expression of the leaderless 730 can only be obtained at very low frequency using a *recA* genetic background (*E. coli* strains: HB101 for cloning; HMS174(DE3) for expression).
- 15
- 20 To overcome this toxicity, a similar approach was used for 730 as described in example 8 for ORF46. Four C-terminal truncated forms were obtained, each of which is well expressed. All were obtained from intracellular expression of His-tagged leaderless 730.

Form A consists of the N-terminal hydrophilic region of the mature protein (aa. 28-226). This was purified as a soluble His-tagged product, having a higher-than-expected MW.

- 25 Form B extends to the end of the region conserved between serogroups (aa. 28-340). This was purified as an insoluble His-tagged product.

The C-terminal truncated forms named C1 and C2 were obtained after screening for clones expressing high levels of 730-His clones in strain HMS174(DE3). Briefly, the pET21b plasmid containing the His-tagged sequence coding for the full-length mature 730 protein was used to transform the *recA* strain HMS174(DE3). Transformants were obtained at low frequency which showed two phenotypes: large colonies and very small colonies. Several large and small colonies were analysed for expression of the 730-His clone. Only cells from large colonies over-expressed a protein recognised by anti-730A antibodies. However the

30

protein over-expressed in different clones showed differences in molecular mass. Sequencing of two of the clones revealed that in both cases integration of an *E. coli* IS sequence had occurred within the sequence coding for the C terminal region of 730. The two integration events have produced in-frame fusion with 1 additional codon in the case of C1, and 12 additional codons in the case of C2 (Figure 11). The resulting "mutant" forms of 730 have the following sequences:

**730-C1 (due to an IS1 insertion - figure 11A)**

	1	MADLAQDPFI	TDNAQRQHYE	PGGKYHLFGD	PRGSVSDRTG	KINVIQDYTH
	51	QMGNLLIQQA	NINGTIGYHT	RFSGHGHEEH	APFDNHAADS	ASEEKGNVDE
10	101	GFTVYRLNWE	GHEHHPADAY	DGPKGGNYPK	PTGARDEYTY	HVNGTARSIK
	151	LNPTDTRSIR	QRISDNYSNL	GSNFSDDRADE	ANRKMFEHNA	KLDRWGNMSME
	201	FINGVAAGAL	NPFISAGEAL	GIGDILYGTR	YAIIDKAAMRN	IAPLPAEGKF
	251	AVIGGLGSVA	GFEKNTREAV	DRWIQENPNA	AETVEAVFNV	AAAAKVAKLA
	301	KAAKPGKAAV	SGDFADSYKK	KLALSDSARQ	LYQNAKYREA	LDIHYEDLIR
15	351	RKTDGSSKFI	NGREIDAVTN	DALIQAR*		

The additional amino acid produced by the insertion is underlined.

**730-C2 (due to an IS5 insertion - Figure 11B)**

	1	MADLAQDPFI	TDNAQRQHYE	PGGKYHLFGD	PRGSVSDRTG	KINVIQDYTH
	51	QMGNLLIQQA	NINGTIGYHT	RFSGHGHEEH	APFDNHAADS	ASEEKGNVDE
20	101	GFTVYRLNWE	GHEHHPADAY	DGPKGGNYPK	PTGARDEYTY	HVNGTARSIK
	151	LNPTDTRSIR	QRISDNYSNL	GSNFSDDRADE	ANRKMFEHNA	KLDRWGNMSME
	201	FINGVAAGAL	NPFISAGEAL	GIGDILYGTR	YAIIDKAAMRN	IAPLPAEGKF
	251	AVIGGLGSVA	GFEKNTREAV	DRWIQENPNA	AETVEAVFNV	AAAAKVAKLA
25	301	KAAKPGKAAV	SGDFADSYKK	KLALSDSARQ	LYQNAKYREA	<u>LGKVRISGEI</u>
	351	<u>LLG</u> *				

The additional amino acids produced by the insertion are underlined.

In conclusion, intracellular expression of the 730-C1 form gives very high level of protein and has no toxic effect on the host cells, whereas the presence of the native C-terminus is toxic. These data suggest that the "intracellular toxicity" of 730 is associated with the C-terminal 65 amino acids of the protein.

Equivalent truncation of ORF29 to the first 231 or 368 amino acids has been performed, using expression with or without the leader peptide (amino acids 1-26; deletion gives cytoplasmic expression) and with or without a His-tag.

**Example 22 - domains in 961**

As described in example 9 above, the GST-fusion of 961 was the best-expressed in *E. coli*. To improve expression, the protein was divided into domains (figure 12).

The domains of 961 were designed on the basis of YadA (an adhesin produced by *Yersinia* which has been demonstrated to be an adhesin localized on the bacterial surface that forms

oligomers that generate surface projection [Hoiczky *et al.* (2000) *EMBO J* 19:5989-99]) and are: leader peptide, head domain, coiled-coil region (stalk), and membrane anchor domain.

These domains were expressed with or without the leader peptide, and optionally fused either to C-terminal His-tag or to N-terminal GST. *E.coli* clones expressing different domains of 961 were analyzed by SDS-PAGE and western blot for the production and localization of the expressed protein, from over-night (o/n) culture or after 3 hours induction with IPTG. The results were:

	Total lysate (Western Blot)	Periplasm (Western Blot)	Supernatant (Western Blot)	OMV SDS-PAGE
961 (o/n)	-	-	-	
961 (IPTG)	+/-	-	-	
961-L (o/n)	+	-	-	+
961-L (IPTG)	+	-	-	+
961c-L (o/n)	-	-	-	
961c-L (IPTG)	+	+	+	
961 $\Delta_1$ -L (o/n)	-	-	-	
961 $\Delta_1$ -L (IPTG)	+	-	-	+

The results show that in *E.coli*:

- 961-L is highly expressed and localized on the outer membrane. By western blot analysis two specific bands have been detected: one at ~45kDa (the predicted molecular weight) and one at ~180kDa, indicating that 961-L can form oligomers. Additionally, these aggregates are more expressed in the over-night culture (without IPTG induction). OMV preparations of this clone were used to immunize mice and serum was obtained. Using overnight culture (predominantly by oligomeric form) the serum was bactericidal; the IPTG-induced culture (predominantly monomeric) was not bactericidal.
- 961 $\Delta_1$ -L (with a partial deletion in the anchor region) is highly expressed and localized on the outer membrane, but does not form oligomers;
- the 961c-L (without the anchor region) is produced in soluble form and exported in the supernatant.

20 Titres in ELISA and in the serum bactericidal assay using His-fusions were as follows:

	ELISA	Bactericidal
961a (aa 24-268)	24397	4096

961b (aa 269-405)	7763	64
961c-L	29770	8192
961c (2996)	30774	>65536
961c (MC58)	33437	16384
961d	26069	>65536

*E.coli* clones expressing different forms of 961 (961, 961-L, 961 $\Delta_1$ -L and 961c-L) were used to investigate if the 961 is an adhesin (*c.f.* YadA). An adhesion assay was performed using (a) the human epithelial cells and (b) *E.coli* clones after either over-night culture or three hours IPTG induction. 961-L grown over-night (961 $\Delta_1$ -L) and IPTG-induced 961c-L (the clones expressing protein on surface) adhere to human epithelial cells.

961c was also used in hybrid proteins (see above). As 961 and its domain variants direct efficient expression, they are ideally suited as the N-terminal portion of a hybrid protein.

### Example 23 – further hybrids

Further hybrid proteins of the invention are shown below (see also Figure 14). These are advantageous when compared to the individual proteins:

#### ORF46.1-741

```

1  ATGTCAGATT TGGCAAACGA TTCTTTTATC CGGCAGGTTC TCGACCGTCA
51  GCATTTTCGAA CCGGACGGGA AATACCACCT ATTTCGGCAGC AGGGGGGAAC
101 TTGCCGAGCG CAGCGGCCAT ATCGGATTGG GAAAAATACA AAGCCATCAG
15 151 TTGGGCAACC TGATGATTCA ACAGGCGGCC ATTAAAGGAA ATATCGGCTA
201 CATTTGTCCGC TTTTCCGATC ACGGGCACGA AGTCCATTCC CCCTTCGACA
251 ACCATGCCTC ACATTCGGAT TCTGATGAAG CCGGTAGTCC CGTTGACGGA
301 TTTAGCCTTT ACCGCATCCA TTGGGACGGA TACGAACACC ATCCCGCCGA
351 CGGCTATGAC GGGCCACAGG GCGGCGGCTA TCCCGCTCCC AAAGGCGCGA
20 401 GGGATATATA CAGCTACGAC ATAAAAGGCG TTGCCCCAAA TATCCGCCTC
451 AACCTGACCG ACAACCGCAG CACCGGACAA CGGCTTGCCG ACCGTTTCCA
501 CAATGCCGGT AGTATGCTGA CGCAAGGAGT AGGCGACGGA TTCAAACGCG
551 CCACCCGATA CAGCCCCGAG CTGGACAGAT CGGGCAATGC CGCCGAAGCC
601 TTCAACGGCA CTGCAGATAT CGTTAAAAAC ATCATCGGCG CGGCAGGAGA
25 651 AATTGTCGGC GCAGCGGATG CCGTGCAGGG CATAAGCGAA GGCTCAAAACA
701 TTGCTGTCAT GCACGGCTTG GGTCTGCTTT CCACCGAAAA CAAGATGGCG
751 CGCATCAACG ATTTGGCAGA TATGGCGCAA CTCAAAGACT ATGCCGCGAG
801 AGCCATCCGC GATTGGGCAG TCCAAAACCC CAATGCCGCA CAAGGCATAG
851 AAGCCGTCAG CAATATCTTT ATGGCAGCCA TCCCATCAA AGGGATTGGA
30 901 GCTGTTTCGGG GAAAATACGG CTGCGGCGGC ATCACGGCAC ATCCTATCAA
951 GCGGTTCGAG ATGGGCGCGA TCGCATTGCC GAAAGGGAAA TCCGCCGTCA
1001 GCGACAATTT TGCCGATGCG GCATACGCCA AATACCCGTC CCCTTACCAT
1051 TCCCGAAATA TCCGTTCAAA CTGAGGAGCAG CGTTACGCGA AAGAAAACAT
1101 CACCTCCTCA ACCGTGCCGC CGTCAAACGG CAAAAATGTC AAAC TGGCAG
35 1151 ACCAACGCCA CCCGAAGACA GGCGTACCGT TTGACGGTAA AGGGTTTCCG
1201 AATTTTGAGA AGCACGTGAA ATATGATACG GGATCCGGAG GGGGTGGTGT
1251 CGCCGCCGAC ATCGGTGCGG GGCTTGCCGA TGCACTAACC GCACCGCTCG
1301 ACCATAAAGA CAAAGGTTTG CAGTCTTTGA CGCTGGATCA GTCCGTCAGG
1351 AAAAACGAGA AACTGAAGCT GGCGGCACAA GGTGCGGAAA AAAC TTATGG
40 1401 AAACGGTGAC AGCCTCAATA CGGGCAAATT GAAGAACGAC AAGGTGAGCC
1451 GTTTCGACTT TATCCGCCAA ATCGAAGTGG ACGGGCAGCT CATTACCTTG
1501 GAGAGTGGAG AGTTCCAAGT ATACAAACAA AGCCATTCCG CCTTAACCGC
1551 CTTTCAGACC GAGCAAATAC AAGATTCCGA GCATTCCGGG AAGATGGTTG
1601 CGAAACGCCA GTTCAGAATC GGCGACATAG CGGGCGAACA TACATCTTTT

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5	1651	GACAAGCTTC	CCGAAGGCGG	CAGGGCGACA	TATCGCGGGA	CGGCGTTCGG
	1701	TTCAGACGAT	GCCGGCGGAA	AACTGACCTA	CACCATAGAT	TTCCGCCGCA
	1751	AGCAGGGAAA	CGGCAAAATC	GAACATTTGA	AATCGCCAGA	ACTCAATGTC
	1801	GACCTGGCCG	CCGCCGATAT	CAAGCCGGAT	GGAAAACGCC	ATGCCGTCAT
	1851	CAGCGGTTCC	GTCCTTTTACA	ACCAAGCCGA	GAAAGGCAGT	TACTCCCTCG
	1901	GTATCTTTGG	CGGAAAAGCC	CAGGAAGTTG	CCGGCAGCGC	GGAAGTGAAA
	1951	ACCGTAAACG	GCATACGCCA	TATCGGCCTT	GCCCGCAAGC	AACTCGAGCA
	2001	CCACCACCAC	CACCACTGA			
10	1	MSDLANDSFI	RQVLDRQHFE	PDGKYHLFGS	RGELAERSGH	IGLGKIQSHQ
	51	LGNLMIQQA	IKGNIGYIVR	FSDHGHEVHS	PFDNHASHSD	SDEAGSPVDG
	101	FSLYRIHWDG	YEHHPADGYD	GPQGGGYAP	KGARDIYSYD	IKGVAQNIRL
	151	NLTDMNRSTGQ	RLADRFHNAG	SMLTQGVGDG	FKRATRYSP	LDRSGNAAEA
	201	FNGTADIVKN	IIGAAGEIVG	AGDAVQGIS	GSNIAMVHGL	GLLSTENKMA
	251	RINDLADMAQ	LKDYAAAAIR	DWAVQNPNA	QGIEAVSNIF	MAAIPKIGIG
	301	AVRGKYGLGG	ITAHPIKRSQ	MGAIALPKGK	SAVSDNFADA	AYAKYPSPYH
15	351	SRNIRSNLEQ	RYGKENITSS	TVPPSNGKNV	KLADQRHPKT	GVPPDGRGFP
	401	NFEKHVKYDT	GSGGGVVAAD	IGAGLADALT	APLDHKDKQL	QSLTLDQSVR
	451	KNEKLKLAQ	GAEKTYGNGD	SLNTGKLNKD	KVSRFDFIRQ	IEVDGQLITL
	501	ESGEFQVYKQ	SHSALTAFQT	EQIQDSEHSG	KMVAKRQFRI	GDIAGEHTSF
	551	DKLPEGGRAT	YRGTAFGSDD	AGGKLTYTID	FAAQKQNGKI	EHLKSPELNV
	601	DLAAADIKPD	GKRHAIVSGS	VLYNQAEKGS	YSLGIFGGKA	QEVAGSAEVK
	651	TVNGIRHIGL	AAKQLEHHHH	HH*		
20						
25						

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	2051	TGAACGCTAC	TACCGAAAAA	TTGGACACAC	GCTTGGCTTC	TGCTGAAAAA
	2101	TCCATTGCCG	ATCACGATAC	TCGCCTGAAC	GGTTTGGATA	AAACAGTGTC
	2151	AGACCTGCGC	AAAGAAACCC	GCCAAGGCCT	TGCAGAACAA	GCCGCGCTCT
5	2201	CCGGTCTGTT	CCAACCTTAC	AACGTGGGTC	GGTTCAATGT	AACGGCTGCA
	2251	GTCGGCGGCT	ACAAATCCGA	ATCGGCAGTC	GCCATCGGTA	CCGGCTTCCG
	2301	CTTTACCGAA	AACTTTGCCG	CCAAAGCAGG	CGTGGCAGTC	GGCACTTCGT
	2351	CCGGTTCTTC	CGCAGCCTAC	CATGTCGGCG	TCAATTACGA	GTGGCTCGAG
	2401	CACCACCACC	ACCACCCTG	A		
10	1	MSDLANDSFI	RQVLDRQHFE	PDGKYHLFGS	RGELAERSGH	IGLGKIQSHQ
	51	LGNLMIQQAA	IKGNIGYIVR	FSDHGHEVHS	PFDNHASHSD	SDEAGSPVDG
	101	FSLYRIHWDG	YEHHPADGYD	GPQGGYPAP	KGARDIYSYD	IKGVAQNIRL
	151	NLTDNRSTGQ	RLADRFHNAG	SMLTQGVGDG	FKRATRYSPE	LDRSGNAAEA
15	201	FNGTADIVKN	IIGAAGEIVG	AGDAVQGISE	GSNIAMVHGL	GLLSTENKMA
	251	RINDLADMAQ	LKDYAAAAIR	DWAVQNPNA	QGIEAVSNIF	MAAIPKIGIG
	301	AVRGKYGLGG	ITAHPIKRSQ	MGAIALPKGK	SAVSDNFADA	AYAKYPSPYH
	351	SRNIRSNLEQ	RYGKENITSS	TVPPSNGKNV	KLADQRHPKT	GVFPDGKFPF
	401	NFEKHVKYDT	SGSGGGATND	DDVKKAATVA	IAAAYNNGQE	INGFKAGETI
20	451	YDIDEDGTIT	KKDATAADVE	ADDFKGLGLK	KVVTNLTKTV	NENKQNVDAK
	501	VKAAESEIEK	LTTKLADTDA	ALADTDAALD	ATTNALNKLK	ENITTFAEET
	551	KTNIVKIDEX	LEAVADTVDK	HAEAFNDIAD	SLDETNTKAD	EAVKTANEAK
	601	QTABETKQNV	DAKVKAETA	AGKAEAAAGT	ANTAADKAEA	VAAKVTDIKA
	651	DIATNKDNIA	KKANSADVYT	REESDSKFVR	IDGLNATTEK	LDTRLASAEK
25	701	SIADHDTRLN	GLDKTVSDLR	KETROGLAEQ	AALSGLFPQY	NVGRFNVTA
	751	VGGYKSES	AVIGTGFRFTE	NFAAKAGVAV	GTSSGSSAAY	HVGVNVEWLE
	801	HHHHHH*				
ORF46.1-961c						
30	1	ATGTCAGATT	TGGCAAACGA	TTCTTTTATC	CGGCAGGTTC	TCGACCGTCA
	51	GCAATTTCGA	CCCAGCGGGA	AATACCACCT	ATTCCGCAGC	AGGGGGGAAC
	101	TTGCCGAGCG	CAGCGGCCAT	ATCGGATTGG	GAAAAATACA	AAGCCATCAG
	151	TTGGGCAACC	TGATGATTCA	ACAGGCGGCC	ATTAAAGGAA	ATATCGGCTA
35	201	CATTGTCCGC	TTTTCGGATC	ACGGGCACGA	AGTCCATTCC	CCCTTCGACA
	251	ACCATGCCTC	ACATTCCGAT	TCTGATGAAG	CCGGTAGTCC	CGTTGACGGA
	301	TTTAGCCTTT	ACCGCATCCA	TTGGGACGGA	TACGAACACC	ATCCCCCGCA
	351	CGGCTATGAC	GGGCCACAGG	GCGGCGGCTA	TCCCCTCCTC	AAAGGCGCGA
	401	GGGATATATA	CAGCTACGAC	ATAAAAGGCG	TTGCCCAAAA	TATCCGCTCG
40	451	AACCTGACCG	ACAACCGCAG	CACCGGACAA	CGGCTTGCCG	ACCGTTTCCA
	501	CAATGCCGGT	AGTATGCTGA	CGCAAGGAGT	AGGCGACGGA	TTCAAACGCG
	551	CCACCCGATA	CAGCCCCGAG	CTGGACAGAT	CGGGCAATGC	CGCCGAAGCC
	601	TTCAACGGCA	CTGCAGATAT	CGTTAAAAAC	ATCATCGGCG	CGGCAGGAGA
	651	AATTGTCCGC	GCAGGCGATG	CCGTGCAGGG	CATAAGCGAA	GGCTCAAACA
45	701	TTGCTGTGTC	GCACGGCTTG	GGTCTGCTTT	CTACCGAAAA	CAAGATGGCG
	751	CGCATCAACG	ATTTGGCAGA	TATGGCGCAA	CTCAAAGACT	ATGCCGCGAG
	801	AGCCATCCGC	GATTGGGCAG	TCCAAAACCC	CAATGCCGCA	CAAGGCATAG
	851	AAGCCGTCAG	CAATATCTTT	ATGGCAGCCA	TCCCCATCAA	AGGGATTGGA
	901	GCTGTTCCGG	GAAAAATACG	CTTGGGCGGC	ATCACGGCAC	ATCCTATCAA
	951	GCGGTCGCAG	ATGGGCGCGA	TGCGATTGCC	GAAAGGGAAA	TCCGCCGTCA
50	1001	GCGACAATTT	TGCCGATGCG	GCATACGCCA	AATACCCGTC	CCCTTACCAT
	1051	TCCCGAAATA	TCCGTTCAAA	CTTGGAGCAG	CGTTACGGCA	AAGAAAACAT
	1101	CACCTCCTCA	ACCGTGCCGC	CGTCAAACGG	CAAAAATGTC	AAACTGGCAC
	1151	ACCAACGCCA	CCCGAAGACA	GGCGTACCGT	TTGACGGTAA	AGGGTTTCCC
55	1201	AATTTTGAGA	AGCACGTGAA	ATATGATACG	GGATCCGGAG	GAGGAGGAGC
	1251	CACAAACGAC	GACGATGTTA	AAAAAGCTGC	CACGTGTGGC	ATTGCTGCTG
	1301	CCTACAACAA	TGGCCAAGAA	ATCAACGGTT	TCAAAGCTGG	AGAGACCATC
	1351	TACGACATTG	ATGAAGACGG	CACAATTACC	AAAAAGACG	CAACTGCAGC
	1401	CGATGTTGAA	GCCGACGACT	TTAAAGGTCT	GGGTCTGAAA	AAAGTCGTGA
	1451	CTAACCTGAC	CAAAACCGTC	AATGAAAACA	AACAAAACGT	CGATGCCAAA
60	1501	GTAAAAGCTG	CAGAATCTGA	AATAGAAAAG	TTAACAACCA	AGTTAGCAGA
	1551	GACTGATGCC	GCTTTAGCAG	ATACTGATGC	CGTCTGGAT	GCAACCACCT
	1601	ACGCCTTGAA	TAAATTGGGA	GAAAATATAA	CGACATTTGC	TGAAGAGACT
	1651	AAGACAAATA	TCGTAAAAAT	TGATGAAAAA	TTAGAAGCCG	TGGCTGATAG
	1701	CGTCGACAAG	CATGCCGAAG	CATTCAACGA	TATCGCCGAT	TCATTGGATG
65	1751	AAACCAACAC	TAAGGCAGAC	GAAGCCGTCA	AAACCGCCAA	TGAAGCCAA
	1801	CAGACGGCCG	AAGAAACCAA	ACAAAACGTC	GATGCCAAAG	TAAAAGCTGC
	1851	AGAAACTGCA	GCAGGCAAAG	CCGAAGCTGC	CGCTGGCACA	GCTAATACTG

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5	1901	CAGCCGACAA	GGCCGAAGCT	GTCGCTGCAA	AAGTTACCGA	CATCAAAGCT
	1951	GATATCGCTA	CGAACAAAGA	TAATATTGCT	AAAAAAGCAA	ACAGTGCCGA
	2001	CGTGACACC	AGAGAAGAGT	CTGACAGCAA	ATTTGTCAGA	ATTGATGGTC
	2051	TGAACGCTAC	TACCGAAAAA	TTGGACACAC	GCTTGGCTTC	TGCTGAAAAA
	2101	TCCATTGCCG	ATCACGATAC	TCGCCTGAAC	GGTTTGGATA	AAACAGTGTC
	2151	AGACCTGCGC	AAAGAAAACC	GCCAAGGCCT	TGCAGAACAA	GCCGCGCTCT
	2201	CCGGTCTGTT	CCAACCTTAC	AACGTGGGTC	TCGAGCACCA	CCACCACCAC
	2251	CACTGA				
10	1	MSDLANDSFI	RQVLDRQHFE	PDGKYHLFGS	RGELAERSGH	IGLGKIQSHQ
	51	LGNLMIQQA	IKGNIGYIVR	FSDHGHEVHS	PFDNHASHSD	SDEAGSPVDG
	101	FSLYRIHWDG	YEHHPADGYD	GPQGGGY PAP	KGARDIYSYD	IKGVAQNIRL
	151	NLTDNRSTGQ	RLADRPHNAG	SMLTQGVGDG	FKRATRYSP	LDRSGNAAEA
	201	FNGTADIVKN	IIGAAGEIVG	AGDAVQGISE	GSNIAMVHGL	GLLSTENKMA
	251	RINDLADMAQ	LKDYAAAAIR	DWAVQNPNA	QGIEAVSNIF	MAAIPKIGIG
	301	AVRGKYGLGG	ITAHPIKRSQ	MGAIALPKGK	SAVSDNFADA	AYAKYPSPYH
15	351	SRNIRSLEQ	RYGKENITSS	TVPPSNGKNV	KLADQRHPKT	GVFPDGGKFP
	401	NFEKHVKYDT	GSGGGGATND	DDVKKAAATVA	IAAAYNNGQE	INGFKAGETI
	451	YDIDEDGTIT	KKDATAADVE	ADDFKGLGLK	KVVTNLTKTV	NENKQNVDAK
	501	VKAAESEIEK	LTTKLADTDA	ALADTDAALD	ATTNALNKLK	ENITTFABET
	551	KTNIVKID EK	LEAVADTVDK	HAEAFNDIAD	SLDETNTKAD	EAVKTANEAK
	601	QTAEETKQNV	DAKVKAAETA	AGKAEAAAGT	ANTAADKAEA	VAAKVTDIKA
	651	DIATNKDNIA	KKANSADVYT	REESDSKFVR	IDGLNATTEK	LDTRLASA EK
20	701	SIADHDTRLN	GLDKTVSDLR	KETRQGLAEQ	AALSGLFQPY	NVGLEHHHHH
	751	H*				

961-ORF46.1

30	1	ATGGCCACAA	ACGACGACGA	TGTTAAAAAA	GCTGCCACTG	TGGCCATTGC
	51	TGCTGCCTAC	AACAATGGCC	AAGAAATCAA	CGGTTTCAA	GCTGGAGAGA
	101	CCATCTACGA	CATTGATGAA	GACGGCACAA	TTACCAAAAA	AGACGCAACT
	151	GCAGCCGATG	TTGAAGCCGA	CGACTTTAAA	GGTCTGGGTC	TGAAAAAAGT
	201	CGTGACTAAC	CTGACCAAAA	CCGTCAATGA	AAACAAACAA	AACGTCGATG
	251	CCAAAGTAAA	AGCTGCAGAA	TCTGAAATAG	AAAAGTTAAC	AACCAAGTTA
35	301	GCAGACACTG	ATGCCGCTTT	AGCAGATACT	GATGCCGCTC	TGGATGCAAC
	351	CACCAACGCC	TTGAATAAAT	TGGGAGAAAA	TATAACGACA	TTTGCTGAAG
	401	AGACTAAGAC	AAATATCGTA	AAAATTGATG	AAAAATTAGA	AGCCGTGGCT
	451	GATACCGTCG	ACAAGCATGC	CGAAGCATTC	AACGATATCG	CCGATTCATT
40	501	GGATGAAACC	AACACTAAGG	CAGACGAAGC	CGTCAAAACC	GCCAATGAAG
	551	CCAAACAGAC	GGCCGAAGAA	ACCAAAACAA	ACGTGCGATC	CAAAGTAAAA
	601	GCTGCAGAAA	CTGCAGCAGG	CAAAGCCGAA	GCTGCCGCTG	GCACAGCTAA
	651	TACTGCAGCC	GACAAGGCCG	AAGCTGTTCG	TGCAAAAGTT	ACCGACATCA
	701	AAGCTGATAT	CGCTACGAAC	AAAGATAATA	TTGCTAAAAA	AGCAAAACAGT
	751	GCCGACGTGT	ACACCAGAGA	AGAGTCTGAC	AGCAAAATTG	TCAGAATTGA
45	801	TGGTCTGAAC	GCTACTACCG	AAAAATTGGA	CACACGCTTG	GCTTCTGCTG
	851	AAAAATCCAT	TGCCGATCAC	GATACTCGCC	TGAACGGTTT	GGATAAAACA
	901	GTGTCTAGACC	TGCCGAAAAG	AACCCGCCAA	GGCCTTGCTG	AACAAGCCGC
	951	GCTCTCCGGT	CTGTTCCAAC	CTTACAACGT	GGGTCGGTTC	AATGTAACGG
	1001	CTGCAGTCGG	CGGCTACAAA	TCCGAATCGG	CAGTCGCCAT	CGGTACCGGC
50	1051	TTCCGCTTTA	CCGAAAACTT	TGCCGCCAAA	GCAGGCGTGG	CAGTCGGCAC
	1101	TTCTGTCGGT	TCTTCCGCG	CCTACCATGT	CGGCGTCAAT	TACGAGTGGG
	1151	GATCCGGAGG	AGGAGGATCA	GATTTGGCAA	ACGATTCTTT	TATCCGGCAG
	1201	GTTCTCGACC	GTCAGCATTT	CGAACCCGAC	GGGAAATACC	ACCTATTCGG
	1251	CAGCAGGGGG	GAACCTTGCCG	AGCGCAGCGG	CCATATCGGA	TTGGGAAAAA
55	1301	TACAAAGCCA	TCAGTTGGGC	AACCTGATGA	TTCAACAGGC	GGCCATTAAA
	1351	GGAAATATCG	GCTACATTGT	CCGCTTTTCC	GATCACGGGC	ACGAAGTCCA
	1401	TTCCCCCTTC	GACAACCATG	CCTCACATTC	CGATTCTGAT	GAAGCCGGTA
	1451	GTCCCGTTGA	CGGATTTAGC	CTTTACCGCA	TCCATTGGGA	CGGATACGAA
	1501	CACCATCCCG	CCGACGGCTA	TGACGGGCCA	CAGGGCGCGC	GCTATCCCGC
60	1551	TCCCAAAGGC	GCGAGGGATA	TATACAGCTA	CGACATAAAA	GGCGTTGCCC
	1601	AAAAATATCCG	CCTCAACCTG	ACCGACAACC	GCAGCACC GG	ACAACGGCTT
	1651	GCCGACCGTT	TCCACAATGC	CGGTAGTATG	CTGACGCAAG	GAGTAGGCGA
	1701	CGGATTCAAA	CGCGCCACCC	GATACAGCCC	CGAGCTGGAC	AGATCGGGCA
	1751	ATGCCGCCGA	AGCCTTCAAC	GGCACTGCAG	ATATCGTTAA	AAACATCATC
65	1801	GGCGCGGCAG	GAGAAATTGT	CGGCGCAGGC	GATGCCGTGC	AGGGCATAAG
	1851	CGAAGGCTCA	AACATTGCTG	TCATGCACGG	CTTGGGTCTG	CTTTCCACCG
	1901	AAAACAAGAT	GGCGCGCATC	AACGATTTGG	CAGATATGGC	GCAACTCAAA

5	1951	GA	CTATG	CCCG	CAGCAGCCAT	CCGCGATTGG	GCAGTCCAAA	ACCCCAATGC
	2001	CG	CACAAGGC	ATAGAAGCCG	TCAGCAATAT	CTTTATGGCA	GCCATCCCCA	
	2051	TC	AAAGGGAT	TGGAGCTGTT	CGGGGAAAAT	ACGGCTTGGG	CGGCATCACG	
	2101	GC	ACATCCTA	TCAAGCGGTC	GCAGATGGGC	GCGATCGCAT	TGCCGAAAGG	
	2151	GAA	ATCCGCC	SKFCAGCACA	ATTTTGCCGA	TGCGGCATAC	GCCAAATACC	
	2201	CG	TCCCCCTTA	CCATTCCCGA	AATATCCGTT	CAAACTTGGA	GCAGCGTTAC	
	2251	GG	CAAAGAAA	ACATCACCTC	CTCAACCGTG	CCGCCGTCAA	ACGGCAAAAA	
	2301	TG	TCAAACCTG	GCAGACCAAC	GCCACCCGAA	GACAGGCGTA	CCGTTTGACG	
10	2351	GT	AAGGGTT	TCCGAATTTT	GAGAAGCACG	TGAAATATGA	TACGCTCGAG	
	2401	CAC	CACCACC	ACCACCCTG	A			
15	1	MAT	NDDDVKK	AATVAIAAAY	NNGQEINGFK	AGETIYDIDE	DGTITKKDAT	
	51	AAD	VEADDFK	GLGLKKVVTN	LTKTVNENKQ	NVDAKVKAEE	SEIEKLTTKL	
	101	AD	TDAALADT	DAALDATFNA	LNKLGENITT	FAEETKTNIV	KIDEKLEAVA	
	151	DT	VDKHAEAF	NDIADSLDET	NTKADBAVKT	ANEAKQTAE	TKQNVDAKVK	
	201	AA	ETAAGKAE	AAAGTANTAA	DKAEEAAKV	TDIKADIATN	KDNIARKANS	
	251	ADV	YTBESD	SKFVRIDGLN	ATTEKLDTRL	ASAEKSIADH	DTRLNGLDKT	
	301	VSD	LRKETRQ	GLAEQAALSG	LFQPYNVGRF	NVTAAVGGYK	SESAVAIGTG	
	351	FR	TENFAAK	AGVAVGTSSG	SSAAYHVGVN	YEWGSGGGGS	DLANDSFIRQ	
20	401	VL	DROHFEPD	GKYHLFGSRG	ELAERSGHIG	LKGIQSHQLG	NLMIQQAAIK	
	451	GN	IYIVRFS	DHGHEVHSPF	DNHASHSDSD	EAGSPVDGFS	LYRIHWDGVE	
25	501	HR	PADYDGP	QGGGYPAKPG	ARDIYSYDIK	GVAQNIRLNL	TDNRSTGQRL	
	551	AD	RFTNAGSM	LTQGVGDGFK	RATRYSPELD	RSAGNIAEFN	GTADIVKNI	
	601	GA	GEIVGAG	DAVQGISSEGS	NIAMVHGLGL	LSTENKMARI	NDLADMAQLK	
	651	DY	AAAIRDW	AVQNPNAAQG	IEAVSNIFMA	APIKGIGAV	RGKYGLGGIT	
	701	AH	PIKRSQMG	ATALPKGKSA	VSDNPFADAA	AKYPSPHYHSR	NIRSNEQRY	
	751	GK	ENITSSTV	PPSNGKNVKL	ADQRHPKTV	PFDGKGFPNF	EKHVKYDTLE	
	801	HH	HHHHH*					
	30	961-741						
1		AT	GGCCACAA	ACGACGACGA	TGTTAAAAAA	GCTGCCACTG	TGGCCATTGC	
35	51	TC	CTGCCTAC	AACAATGGCC	AAGAAATCAA	CGGTTTCAAA	GCTGGAGAGA	
	101	CC	ATCTAGCA	CATTGATGAA	GACGGCACTA	TTACCAAAAA	AGACGAGAGT	
	151	GC	AGCCGATG	TGAAGCCGA	CGACTTTAAA	GGTCTGGGTC	TGAAAAAAGT	
	201	CG	TGACTAAC	CTGACCAAAA	CCGTCAATGA	AAACAAACAA	AACGTCGATG	
	251	CC	AAAGTAAA	AGCTGCAGAA	TCTGAAATAG	AAAAGTTAAC	AACCAAGTTA	
	301	GC	AGACACTG	ATGCCGCTTT	AGCAGATACT	GATGCCGCTC	TGGATGCAAC	
	351	CAC	CAACGCC	TTGAATAAAT	TGGGAGAAAA	TATAACGACA	TTTGCTGAAG	
	401	AG	ACTAAGAC	ATTAATCGTA	AAAATTGATG	AAAAAATTAGA	AGCCGTGGCT	
40	451	GAT	ACCGTCG	ACAAGCATGC	CGAAGCATT	AACGATATCG	CCGATTTCATT	
	501	GG	ATGAAACC	AACACTAAGG	CAGACGAAGC	CGTCAAAACC	GCCAATGAAG	
	551	CC	AAACAGAC	GGCCGAAGAA	ACCAACACAA	ACGTCGATGC	CAAAGTAAAA	
	601	GCT	GCAGAAA	CTGCAGCAGG	CAAAGCCGAA	GCTGCCGCTG	GCACAGCTAA	
	651	TAC	TGCAGCC	GACAAGGCCG	AAGCTGCTCG	TGCATAAAGTT	ACCGACATCA	
	701	AAG	CTGATAT	CGCTACGAAC	AAAGATAATA	TTGCTAAAAA	AGCAAACAGT	
	751	GCC	GACGTGT	ACACCAGAGA	AGAGTCTGAC	AGCAAATTTG	TCAGAATTGA	
	801	TGG	TCTGAAC	GCTACTACCG	AAAAATTGGA	CACACGCTTG	GCTTCTGCTG	
50	851	AAAA	ATCCATT	TGCCGATCAC	GATACTCGCC	TGAACGGTGT	GGATAAAAA	
	901	GT	GTACAGAC	TGCCGATACA	AACCCGCCAA	GGCCTTGCAG	AACAAGCCGC	
	951	GCT	CTCCGGT	CTGTTCCAAC	CTTACAACGT	GGTTCGGTTC	AATGTAACCG	
	1001	CT	GCACTCG	CGGCTACAAA	TCCGAATCGG	CAGTCGCCAT	CGGTACCGGC	
	1051	TT	CCGCTTTA	CCGAAAACCT	TGCCGCCAAA	GCAGGCGTGG	CAGTCGGCAC	
	1101	TT	CGTCCGGT	TCCTCCGCAG	CCTACCATGT	CGGCGTCAAT	TACGATGGGG	
	1151	GAT	CCCGGAG	GGGTGGTGT	GCCGCCGACA	TCGGTTCGGG	GCTTGGCCAT	
	1201	GC	ATTAACCG	CAACCGTCGA	CCATAAAGAC	AAAGGTTTGC	AGTCTTTGAC	
55	1251	GCT	GGATCAG	TCCGTCAGGA	AAAACAGGAA	ACTGAAGCTG	CGGGCACAAG	
	1301	GT	GCGGAAAA	AACCTTATGGA	AACGGTGACA	GCCTCAATAC	GGGCAAATTG	
	1351	AAG	AACGACA	AGGTCAAGCCG	TTTTCGACTTT	ATCCGCCAAA	TCGAAGTGGA	
	1401	CG	GGCAGCTC	ATTACCTTGG	AGAGTGAGGA	GTTCACAGTA	TACAAACAAA	
	1451	GCA	ATTCCTCG					

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	1851	CGGCAGCGCG	GAAGTGA AAAA	CCGTAAACGG	CATACGCCAT	ATCGGCCTTG
	1901	CCGCCAAGCA	ACTCGAGCAC	CACCACCACC	ACCACTGA	
5	1	MATNDDDVKK	AATVAIAAAY	NNGQEINGFK	AGETIYDIDE	DGTITKKDAT
	51	AADVEADDFK	GLGLKKVVTN	LTKTVNENKQ	NVDAKVKAEE	SEIEKLTTKL
	101	ADTDAALADT	DAALDATNA	LNKLGENITT	FAEETKTIV	KIDEKLEAVA
	151	DTVDKHAEAF	NDIADSLDET	NTKADEAVKT	ANEAKQTAEE	TKQNVDAKVK
	201	AAETAAGKAE	AAAGTANTAA	DKAEAVA AAKV	TDIKADIATN	KDNIARKANS
10	251	ADVYTREESD	SKFVRIDGLN	ATTEKLDTRL	ASAEKSIADH	DTRLNGLDKT
	301	VSDLRKETRQ	GLAEQAALSG	LFQPVNVGRF	NVTAAVGGYK	SESAVAIGTG
	351	FRFTENFAAK	AGVAVGTSSG	SSAAHVGVN	YEWGSGGGGV	AADIGAGLAD
	401	ALTAFLDHKD	KGLQSLTLDQ	SVRKNEKLKL	AAQGAERTYG	NGDSLNTGKL
	451	KNDKVSRLFDF	IRQIEVDGQL	ITLESGEFQV	YKQSHSALTA	FQTEBQIQDSE
15	501	HSGKMVAKRQ	FRIGDIAGEH	TSFDKLPEGG	RATYRGTAFG	SDDAGGKLTLY
	551	TIDFAAKQGN	GKIEHLKSPE	LNVDLAAADI	KPDGKRHAVI	SGSVLYNQAE
	601	KGSYSLGIFG	GKAQEVAGSA	EVKTVNGIRH	IGLAAKQLEH	HHHHH*
20	961-983					
	1	ATGGCCACAA	ACGACGACGA	TGTTAAAAAA	GCTGCCACTG	TGGCCATTGC
	51	TGCTGCCTAC	AACAATGGCC	AAGAAATCAA	CGGTTTCAAA	GCTGGAGAGA
	101	CCATCTACGA	CATTGATGAA	GACGGCACAA	TTACCAAAAA	AGACGCAACT
	151	GCAGCCGATG	TTGAAGCCGA	CGACTTTAAA	GGTCTGGGTC	TGAAAAAAGT
25	201	CGTGACTAAC	CTGACCAAAA	CCGTCAATGA	AAACAACAAA	AACGTCGATG
	251	CCAAAGTAAA	AGCTGCAGAA	TCTGAAATAG	AAAAGTTAAC	AACCAAGTTA
	301	GCAGACACTG	ATGCCGCTTT	AGCAGATACT	GATGCCGCTC	TGGATGCAAC
	351	CACCAACGCC	TTGAATAAAT	TGGGAGAAAA	TATAACGACA	TTTGCTGAAG
	401	AGACTAAGAC	AAATATCGTA	AAAATTGATG	AAAAATTAGA	AGCCGTGGCT
30	451	GATACCGTCG	ACAAGCATGC	CGAAGCATTC	AACGATATCG	CCGATTCTATT
	501	GGATGAAACC	AACACTAAGC	CAGACGAAGC	CGTCAAAACC	GCCAAATGAAG
	551	CCAAACAGAC	GGCCGAAGAA	ACCAAAACAA	ACGTCGATGC	CAAAGTAAAA
	601	GCTGCAGAAA	CTGCAGCAGG	CAAAGCCGAA	GCTGCCGCTG	GCACAGCTAA
	651	TACTGCAGCC	GACAAGGCCG	AAGCTGTGCG	TGCAAAAGTT	ACCGACATCA
35	701	AAGCTGATAT	CGCTACGAAC	AAAGATAATA	TTGCTAAAAA	AGCAAACAGT
	751	GCCGAGCTGT	ACACCAGAGA	AGAGTCTGAC	AGCAAATTTG	TCAGAAATTGA
	801	TGGTCTGAAC	GCTACTACCG	AAAAATTGGA	CACACGCTTG	GCTTCTGCTG
	851	AAAAATCCAT	TGCCGATCAC	GATACTCGCC	TGAACGGTTT	GGATAAAACA
	901	GTGTCAGACC	TGCGCAAAGA	AACCCGCCAA	GGCCTTGACG	AACAAGCCGC
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50	1451	GCGGGGTAGA	GGTAGGTATC	GTCGACACAG	GCGAATCCGT	CGGCAGCATA
	1501	TCCTTTCCCG	AACTGTATGG	CAGAAAAGAA	CACGGCTATA	ACGAAAATTA
	1551	CAAAAACATAT	ACGGCGTATA	TGCGGAAGGA	AGCGCCTGAA	GACGGAGGCG
	1601	GTAAAGACAT	TGAAGCTTCT	TTTCGACGATG	AGGCCGTTAT	AGAGACTGAA
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	1801	AAGAACGAAA	TGATGGTTGC	AGCCATCCGC	AATGCATGGG	TCAAGCTGGG
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	2001	CCTGATGCAA	CAGAGCGATT	ACGGCAACCT	GTCCTACCAC	ATCCGTAAATA
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	2301	TTTCACCCGT	ACAAACCCGA	TTCAAATTTG	CGGAACATCT	TTTTCGCCAC
	2351	CCATCGTAAC	CGGCACGGCG	GCTCTGCTGC	TGCAGAAATA	CCCGTGGATT

5	2401	AGCAACGACA	ACCTGCGTAC	CACGTTGCTG	ACGACGGCTC	AGGACATCGG
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	2501	AGGCCATGAA	CGGACCCGCG	TCCTTTCCGT	TCGGCGACTT	TACCGCCGAT
	2551	ACGAAAGGTA	CATCCGATAT	TGCCTACTCC	TTCCGTAACG	ACATTTCAGG
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	3251	TGGATGCCCT	CGAATCATCC	GCAACACCCG	AGACGGTTGA	AACTGCGGCA
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	4251	GCGCGGTTCC	AAACAGTACG	GCAACCACAG	CGGACGAGTC	GGCGTAGGCT
	4301	ACCGGTTCTT	CGAGCACCAC	CACCACCACC	ACTGA	
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	151	DTVVDKHAFA	NDIADSLDET	NTKADEAVKT	ANEAKQTAE	TKQNVDAVK
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	351	FRFTENFAAK	AGVAVGTSSG	SSAAYHVGUN	YEWGSGGGGT	SAPDFNAGGT
	401	GIGNSRATT	AKSAAVSYAG	IKNEMCKDRS	MLCAGRDDVA	VTDRDAKINA
	451	PPPNLHTGDF	PNPNDAYKNL	INLKPAIEAG	YTGRGVEVGI	VDTGESVSGI
	501	SFPPLYGRKE	HGYNENYKNY	TAYMRKEAPE	DGGGKDIEAS	FDDEAVIETE
55	551	AKPTDIRHVK	EIGHIDLVS	IIGGRSVDGR	PAGGIAPDAT	LHIMNTNDET
	601	KNEMMVAAIR	NAWVKLGERG	VRIVNNSFGT	TSRAGTADLF	QIANSEEQYR
	651	QALLDYSGGD	KTDEGIRLMQ	QSDYGNLSYH	IRNKNMLFIF	STGNDAQAP
	701	NTYALLPFYE	KDAQKGITTV	AGVDRSGEKF	KREMYGEPGT	EPLEYGSNHC
	751	GITAMWCLSA	PYEASVRFT	TNPIQIAGTS	FSAPIVTGTA	ALLLQKYPWM
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	851	TKGTSDIAYS	FRNDISGTGG	LIKKGGSQLQ	LHGNNYTGK	TIIEGGSVL
	901	YGNKSDMRV	ETKALYING	AASGGSLSND	GIVYLADTDQ	SGANETVHIK
	951	GSLQLDGKGT	LYTRLGKLLK	VDGTALIGGK	LYMSARGKGA	GYNLSTGRRV
	1001	PFLSAAKIGQ	DYSFFTNIET	DGGLLASLDS	VEKTAGSEGD	TLSYVVRGRN
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	1101	ADRTDMPGIR	PYGATFRAAA	AVQHANAADG	VRIFNSLAAT	VYADSTAHA
	1151	DMQGRRLKAV	SDGLDHNGTG	LRVIAQTQQD	GGTWEQGGVE	GKMRGSTQTV
	1201	GIAAKTGENT	TAAATLGMGR	STWSENSANA	KTDSISLFA	IRHDAGDIGY
	1251	LKGLFSYGRY	KNSISRSTGA	DEHAEGSVNG	TLMQLGALGG	VNVFFAATGD
	1301	LTVEGGLRYD	LLKQDAFAEK	GSALGWSGNS	LTEGTLVGLA	GLKLSQPLSD

1351 KAVLFATAGV ERDLNGRDYT VTGGFTGATA ATGKTGARNM PHTRLVAGLG  
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751 H\*

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10	251	CCAAAGTAAA	AGCTGCAGAA	TCTGAAATAG	AAAAGTTAAC	AACCAAGTTA
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	301	VSDLRKETRQ	GLAEQAALSG	LFQPNVNGGS	GGGVAAADIG	AGLADALTAP
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	501	AKQGNKIEH	LKSPELNVDL	AAADIKPDGK	RHAVISGSVL	YNQAERKSYS
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751 H\*

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 551 CCAAACAGAC GGCCGAAGAA ACCAAACAAA ACGTCGATGC CAAAGTAAAA  
 601 GCTGCAGAAA CTGCAGCAGG CAAAGCCGAA GCTGCCGCTG GCACAGCTAA  
 651 TACTGCAGCC GACAAGGCCG AAGCTGTGCG TGCAAAAGTT ACCGACATCA  
 701 AAGCTGATAT CGCTACGAAC AAAGATAATA TTGCTAAAAA AGCAAAAGT  
 20 751 GCCGACGTGT ACACCAGAGA AGAGTCTGAC AGCAAATTTG TCAGAATTGA  
 801 TGGTCTGAAC GCTACTACCG AAAAATTGGA CACACGCTTG GCTTCTGCTG  
 851 AAAAATCCAT TGCCGATCAC GATACTCGCC TGAACGGTTT GGATAAAACA  
 901 GTGTCAGACC TCGCAGAAAG AACC CGCCAA GGCCTTGCAG AACAAAGCCG  
 951 GCTCTCCGGT CTGTTCCAAC CTTACAACGT GGGTGGATCC GGAGGGGGTG  
 25 1001 GTGTCGCCGC CGACATCGGT GCGGGGCTTG CCGATGCACT AACCGCACCG  
 1051 CTCGACCATA AAGACAAAGG TTTGCAGTCT TTGACGCTGG ATCAGTCCGT  
 1101 CAGGAAAAAC GAGAACTGA AGCTGGCGGC ACAAGGTGCG GAAAAAATT  
 1151 ATGGAACCGG TGACAGCCTC AATACGGGCA AATTGAAGAA CGACAAGGTC  
 1201 AGCCGTTTCG ACTTTATCCG CCAAATCGAA GTGGACGGGC AGCTCATTAC  
 30 1251 CTTGGAGAGT GGAGAGTTC AAGTATACAA ACAAAGCCAT TCCGCTTAA  
 1301 CCGCTTTCA GACCGAGCAA ATACAAGATT CGGAGCATTC CGGGAAGATG  
 1351 GTTGCGAAAC GCCAGTTCAG AATCGGCGAC ATAGCGGGCG AACATACATC  
 1401 TTTTGACAAG CTTCCCGAAG GCGGCAGGGC GACATATCGC GGGACGGCGT  
 1451 TCGGTTTCAGA CGATGCCGGC GGAAACTGA CCTACACCAT AGATTTCCGC  
 35 1501 GCCAAGCAGG GAAACGGCAA AATCGAACAT TTGAAATCGC CAGAACTCAA  
 1551 TGTCGACCTG GCCGCCGCCG ATATCAAGCC GGATGGAAAA CGCATGCCC  
 1601 TCATCAGCGG TTCCGTCCCT TACAACCAAG CCGAGAAAGG CAGTTACTCC  
 1651 CTCGGTATCT TTGGCGGAAA AGCCCAGGAA GTTGCCGGCA GCGCGAAGT  
 1701 GAAAACCGTA AACGGCATA GGCATATCGG CCTTGCCGCC AAGCAACTCG  
 40 1751 AGCACCACCA CCACCACCAC TGA

1 MATNDDVKK AATVAIAAAY NNGQEINGFK AGETIYDIDE DGTITKKDAT  
 51 AADVEADDFK GLGLKVVVN LTKTVNENKQ NVDAKVKAEE SEIEKLTTKL  
 101 ADTDAALADT DAALDATNNA LNKLGENTIT FAETKTNIV KIDEKLEAVA  
 45 151 DTVDKHAEAF NDIADSLDET NTKADEAVKT ANEAKQTAEE TKQNVDAKVK  
 201 AAEATAAGKAE AAAGTANTAA DKAEEVAARKV TDIKADIATN KDNIAKKANS  
 251 ADVYTREESD SKFVRIDGLN ATTEKLDTRL ASAEKSIADH DTRLNGLDKT  
 301 VSDLRKETRQ GLAEQAALSG LFPYNVGGS GGGGVAADIG AGLADALTAP  
 351 LDHKDKGLQS LTLDSVVRKN EKLKLAQGA EKTYNGDSL NTGKLKNDKV  
 50 401 SRFD FIRQIE VDGQLITLES GEFOVYKQSH SALTAFOTEQ IQDSEHSGKM  
 451 VAKRQFRIGD IAGEHTSFDK LPEGGRATYR GTAFGSDDAG GKLTYYTIDFA  
 501 AKQNGKIEH LKSPELNVDL AAADIKPDGK RHAVISGSVL YNQAERKGSYS  
 551 LGIFGGKAQE VAGSAEVKTV NGIRHIGLAA KQLEHHHHHH \*

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55 1 ATGGCCACAA ACGACGACGA TGTTAAAAAA GCTGCCACTG TGGCCATTGC  
 51 TGCTGCCTAC AACAATGGCC AAGAAATCAA CGGTTTCAAA GCTGGAGAGA  
 60 101 CCATCTACGA CATTGATGAA GACGGCACAA TTACCAAAAA AGACGCAACT  
 151 GCAGCCGATG TTGAAGCCGA CGACTTTAAA GGTCTGGGTC TGAAAAAAGT  
 201 CGTGACTAAC CTGACCAAAA CCGTCAATGA AAACAAACAA AACGTCGATG  
 251 CCAAAGTAAA AGCTGCAGAA TCTGAAATAG AAAAGTTAAC AACCAAGTTA  
 301 GCAGACACTG ATGCCGCTTT AGCAGATACT GATGCCGCTC TGGATGCAAC  
 351 CACCAACGCC TTGAATAAAT TGGGAGAAAA TATAACGACA TTTGCTGAAG  
 65 401 AGACTAAGAC AAATATCGTA AAAATTGATG AAAAATTAGA AGCCGTGGCT  
 451 GATACCGTCG ACAAGCATGC CGAAGCATTC AACGATATCG CCGATTCAAT  
 501 GGATGAAACC AACACTAAGG CAGACGAAGC CGTCAAAACC GCCAATGAAG

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3901	GCAACGGCGG	GCGTGGAACG	CGACCTGAAC	GGACGCGACT	ACACGGTAAC
3951	GGGCGGCTTT	ACCGGCGCGA	CTGCAGCAAC	CGGCAAGACG	GGGGCACGCA
4001	ATATGCCGCA	CACCCGCTCTG	GTGCGCGGCC	TGGGCGCGGA	TGTCGAATTC
4051	GGCAACGGCT	GGAACGGCTT	GGCACGTTAC	AGCTACGCCG	GTTCCAAACA
4101	GTACGGCAAC	CACAGCGGAC	GAGTCGGCGT	AGGCTACCGG	TTCTCTGAGC
4151	ACCACCACCA	CCACCACTGA			
1	MATNDDDVKK	AATVALAAAY	NNGQEINGFK	AGETIYDIDE	DGTITKKDAT
51	AADVEADDFK	GLGLKKVVTN	LTKTVNENKQ	NVDKVKAAE	SEIEKLTTKL
101	ADTDAALADT	DAALDATTNA	LNKLGENITT	FAEETKTNIV	KIDEKLEAVA
151	DTVDKHAEAF	NDIADSLDET	NTKADEAVKT	ANEAKQTAE	TKQNVDAKVK
201	AAETAAGKAE	AAAGTANTAA	DKAEAVAAKV	TDIKADIATN	KDNIAKKANS
251	ADVYTREESD	SKFVRIDGLN	ATTEKLDTRL	ASAEKSIADH	DTRLNGLDKT
301	VSDLRKETRQ	GLAEQAALSG	LFQPYNVGGS	GGGGTSAPDF	NAGGTGIGSN
351	SRATTAKSAA	VSAGIKNEM	CKDRSMLCAG	RDDVAVTDRD	AKINAPPPNL
401	HTGDFPNPND	AYKNLINLKP	AIEAGYTGRG	VEVGIVDTGE	SVGSISFPPEL
451	YGRKEHGYNE	NYKNYTAYMR	KEAPEDEGGK	DIEASFDDDEA	VIETEAAPTD
501	IRHVKEIGHI	DLVSHIIGGR	SVDGRFAGGI	APDATLHIMN	TNDETKNEMM
551	VAAIRNAWVK	LGERGVRIVN	NSFGTTSRAG	TADLFQIANS	EEQYRQALLD
601	YSGGDKTDEG	IRLMQQSDYG	NLSYHIRNKN	MLFIFSTGND	AQAQPNITYAL
651	LPFYEKDAQK	GIITVAGVDR	SGEKFKREMY	GEPGTEPLEY	GSNHCGITAM
701	WCLSAPYEAS	VRFTRTNPIQ	IAGTSFSAPI	VTGTAALLLQ	KYPWMSNDNL
751	RTLLTQAQD	IGAVGVDSKF	GWGLLDAGKA	MNGPASFPFG	DFTADTKGTS
801	DIAYSFRNDI	SGTGGLIKKG	GSQQLHGN	TYTGKTIIEG	GSLVLVGNK
851	SDMRVETKGA	LIYNGAASGG	SLNSDGIIVL	ADTDQSGANE	TVHIKGSLLQ
901	DGKGTLYTRL	GKLLKVDGTA	IIGGKLYMSA	RGKGAGYLS	TGRRVPFLSA
951	AKIGQDYSFF	TNIETDGGLL	ASLDSVEKTA	GSEGDLSY	VRRGNAARTA
1001	SAAAHSAAPAG	LKHAQQGGS	NLENLMVELD	ASESSATPET	VETAAADRTD
1051	MPGIRPYGAT	FRAAAAVQHA	NAADGVRIFN	SLAATVYADS	TAAHADMQGR
1101	RLKAVSDGLD	HNGTGLRVIA	QTQDGGTWE	QGGVEGKMRG	STQTVGIAAK
1151	TGENTTAAAT	LGMGRSTWSE	NSANAKTDSI	SLFAGIRHDA	GDIGYLKGLF
1201	SYGRYKNSIS	RSTGADEHAE	GSVNGTLMQL	GALGGVNVPF	AATGDLTVEG
1251	GLRYDLLKQD	AFAEKGSALG	WSGNSLTEGT	LVGLAGLKLS	QPLSDKAVLF
1301	ATAGVERDLN	GRDYTVTGGF	TGATAATGKT	GARNMPHTRL	VAGLGADVEF
1351	GNGWNLARY	SYAGSKQYGN	HSGRVGVGYR	FLEHHHHHH*	

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1	ATGAAACACT	TTCCATCCAA	AGTACTGACC	ACAGCCATCC	TTGCCACTTT
51	CTGTAGCGGC	GCACTGGCAG	CCACAAACGA	CGACGATGTT	AAAAAAGCTG
101	CCACTGTGGC	CATTGCTGCT	GCCTACAACA	ATGGCCAAGA	AATCAACGGT
151	TTCAAAGCTG	GAGAGACCAT	CTACGACATT	GATGAAGACG	GCACAATTAC
201	CAAAAAAGAC	GCAACTGCAG	CCGATGTTGA	AGCCGACGAC	TTTAAAGGTC
251	TGGGTCTGAA	AAAAGTCGTG	ACTAACCTGA	CCAAAACCGT	CAATGAAAAC
301	AAACAAAACG	TCGATGCCAA	AGTAAAAGCT	GCAGAATCTG	AAATAGAAAA
351	GTTAACAACC	AAGTTAGCAG	ACACTGATGC	CGCTTTAGCA	GATACTGATG
401	CCGCTCTGGA	TGCAACCACC	AACGCCCTGA	ATAAATTGGG	AGAAAATATA
451	ACGACATTTG	CTGAAGAGAC	TAAGACAAAT	ATCGTAAAAA	TTGATGAAAA
501	ATTAGAAGCC	GTGGCTGATA	CCGTCGACAA	GCATGCCGAA	GCATTCAACG
551	ATATCGCCGA	TTCAATTGGAT	GAAACCAACA	CTAAGGCAGA	CGAAGCCGTC
601	AAAACCGCCA	ATGAAGCCAA	ACAGACGGCC	GAAGAAACCA	AACAAAACGT
651	CGATGCCAAA	GTAAGAGCTG	CAGAACTGCG	AGCAGGCAAA	GCCGAAGCTG
701	CCGCTGGCAC	AGCTAATACT	GCAGCCGACA	AGGCCGAAGC	TGTCGCTGCA
751	AAAGTTACCG	ACATCAAAGC	TGATATCGCT	ACGAACAAAG	ATAATATTGC
801	TAAAAAAGCA	AACAGTGCCG	ACGTGTACAC	CAGAGAAGAG	TCTGACAGCA
851	AATTTGTCTAG	AATTGATGGT	CTGAACGCTA	CTACCGAAAA	ATTGGACACA
901	CGCTTGGCTT	CTGCTGAAAA	ATCCATTGCC	GATCACGATA	CTCGCCTGAA
951	CGGTTTGGAT	AAAACAGTGT	CAGACCTGCG	CAAAGAAACC	CGCCAAGGCC
1001	TTGCAGAAC	AGCCGCGCTC	TCCGGTCTGT	TCCAACCTTA	CAACGTGGGT
1051	GATCCGGAG	GAGGAGGATC	AGATTGGCA	AACGATTCTT	TTATCCGGCA
1101	GGTTCTCGAC	CGTCAGCATT	TCGAACCCGA	CGGGAAATAC	CACCTATTCCG
1151	GCAGCAGGGG	GGAACCTGCC	GAGCGCAGCG	GCCATATCGG	ATTGGGAAAA
1201	ATACAAAGCC	ATCAGTTGGG	CAACCTGATG	ATTCAACAGG	CGGCCATTAA
1251	AGGAAATATC	GGCTACATTG	TCCGCTTTTC	CGATCACGGG	CACGAAGTCC
1301	ATTCCCCCTT	CGACAACCAT	GCCTCACATT	CCGATTCTGA	TGAAGCCGGT
1351	AGTCCCGTTG	ACGGATTTAG	CCTTTACCGC	ATCCATTGGG	ACGGATACGA
1401	ACACCATCCC	GCCGACGGCT	ATGACGGGCC	ACAGGGCGGC	GGCTATCCCC

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5	1451	CTCCCAAAGG	CGCGAGGGAT	ATATACAGCT	ACGACATAAA	AGGCGTTGCC
	1501	CAAAATATCC	GCCTCAACCT	GACCGACAAC	CGCAGCACCG	GACAACGGCT
	1551	TGCCGACCGT	TTCCACAATG	CCGGTAGTAT	GCTGACGCAA	GGAGTAGGCG
	1601	ACGGATTCAA	ACGCGCCACC	CGATACAGCC	CCGAGCTGGA	CAGATCGGGC
	1651	AATGCCGCGG	AAGCCTTCAA	CGGCACTGCA	GATATCGTTA	AAAACATCAT
	1701	CGGCGCGGCA	GGAGAAATTG	TCGGCGCAGG	CGATGCCGTG	CAGGCATAAA
	1751	GCGAAGGCTC	AAACATTGCT	GTCATGCACG	GCTTGGGTCT	GCTTTCCACC
	1801	GAAAACAAGA	TGGCGCGCAT	CAACGATTTG	GCAGATATGG	CGCAACTCAA
10	1851	AGACTATGCC	GCAGCAGCCA	TCCGCGATTG	GGCAGTCCAA	AACCCCAATG
	1901	CCGCACAAGG	CATAGAAGCC	GTCAGCAATA	TCTTTATGGC	AGCCATCCCC
	1951	ATCAAAGGGA	TTGGAGCTGT	TCGGGGAAAA	TACGGCTTGG	GCGGCATCAC
	2001	GGCACATCCT	ATCAAGCGGT	CGCAGATGGG	CGCGATCGCA	TTGCCGAAAG
15	2051	GGAAATCCGC	CGTCAGCGAC	AATTTTGCCG	ATGCGGCATA	CGCCAAATAC
	2101	CCGTCCCCCT	ACCATTTCCG	AAATATCCGT	TCAAACCTGG	AGCAGCGTTA
	2151	CGGCAAAGAA	AACATCACCT	CCTCAACCGT	GCCGCCGTCA	AACGGCAAAA
	2201	ATGTCAAAC	GGCAGACCAA	CGCCACCCGA	AGACAGGCGT	ACCGTTTGAC
	2251	GGTAAAGGGT	TTCCGAATTT	TGAGAAGCAC	GTGAAATATG	ATACGTAAC
	2301	CGAG				
20						
25	1	MKHFP SKVLT	TAILATFCSG	ALAATNDDDV	KKAATVAIAA	AYNNGQEING
	51	FKAGETIYDI	DEDGTITKKD	ATAADVEADD	FKGLGLKKVV	TNLTKTVNEN
	101	KQNVDAKVA	AESIEIKLTT	KLADTDAALA	DTDAALDAT	NALNLKGENI
	151	TTFAEETKTN	IVKIDKLEA	VADTVDKHAE	AFNDIADSLD	ETNTRADEAV
	201	KTANEAKQTA	EETKQNVDAK	VKAAETAAGK	AEAAAGTANT	AADKAEAVAA
	251	KVTDIKADIA	TNKDNIARKA	NSADVYTREE	SDSKFVRIDG	LNATTEKLD
	301	RLASAERSTIA	DHDTRLNLGLD	KTVSDLRKET	RQGLAEQAL	SGLFQPNVNG
	351	GSGGGGSDLA	NDSFIRQVLD	RQHFEPDGKY	HLFGSRGELA	ERSGHIGLGK
30	401	IQSHQLGNLM	IQQAAIKGNI	GYIVRFS DHG	HEVHSPFDNH	ASHSDSDEAG
	451	SPVDGFSLYR	IHWGDYEHHP	ADGYDGPQGG	GYPAPKGARD	IYSYDIKQVA
	501	QNIRLNLTDN	RSTGQRLADR	FHNAGSMLTQ	GVGDGFKRAT	RYSPELDRSG
	551	NAAEAFNGTA	DIVKNIIGAA	GEIVGAGDAV	QGISSEGSNIA	VMHGLGLLST
	601	ENKMARINDL	ADMAQLKDYA	AAAIRDWAVQ	NPNAAQGIEA	VSNIFMAAIP
	651	IKGIGAVRGK	YGLGGITAHF	IKRSQMGALA	LPGKSAVSD	NFADAAYAKY
	701	PSPYHSRNR	SNLEQRYGKE	NITSSTVPPS	NGKNVKLADQ	RHPKTVGVPD
	751	GKGFNPFKX	VKYDT*			
35						

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40	1	ATGAAACACT	TTCCATCCAA	AGTACTGACC	ACAGCCATCC	TTGCCACTTT
	51	CTGTAGCGGC	GCACTGGCAG	CCACAAACGA	CGACGATGTT	AAAAAGCTG
	101	CCACTGTGGC	CATTGCTGCT	GCCTACAACA	ATGGCCAAGA	AATCAACGGT
	151	TTCAAAGCTG	GAGAGACCAT	CTACGACATT	GATGAAGACG	GCACAATTAC
	201	CAAAAAAGAC	GCAACTGCAG	CCGATGTTGA	AGCCGACGAC	TTTAAAGGTC
45	251	TGGGTCTGAA	AAAAGTCGTG	ACTAACCTGA	CCAAAACCGT	CAATGAAAAAC
	301	AAACAAAACG	TCGATGCCAA	AGTAAAAGCT	GCAGAATCTG	AAATAGAAAA
	351	GTTAACAACC	AAGTTAGCAG	ACACTGATGC	CGCTTTAGCA	GATACTGATG
	401	CCGCTCTGGA	TGCAACCACC	AACGCCTTGA	ATAAATTGGG	AGAAAATATA
	451	ACGACATTTG	CTGAAGAGAC	TAAGACAAAT	ATCGTAAAAA	TTGATGAAAA
50	501	ATTAGAAGCC	GTGGCTGATA	CCGTGACAAA	GCATGCCGAA	GCATTCAACG
	551	ATATCGCCGA	TTCAATTGGAT	GAAACCAACA	CTAAGGCAGA	CGAAGCCGTC
	601	AAAACCGCCA	ATGAAGCCAA	ACAGACGGCC	GAAGAAACCA	AACAAAACGT
	651	CGATGCCAAA	GTAAAAGCTG	CAGAAACTGC	AGCAGGCAAA	GCCGAAGCTG
	701	CCGCTGGCAC	AGCTAATACT	GCAGCCGACA	AGGCCGAAGC	TGTCGCTGCA
	751	AAAGTTACCG	ACATCAAAGC	TGATATCGCT	ACGAACAAAG	ATAATATTGC
55	801	TAAAAAAGCA	AACAGTGCCG	ACGTGTACAC	CAGAGAAGAG	TCTGACAGCA
	851	AATTTGTGAG	AATTGATGGT	CTGAACGCTA	CTACCGAAAA	ATTGGACACA
	901	CGCTTGGCTT	CTGCTGAAAA	ATCCATTGCC	GATCACGATA	CTCGCTTGAA
	951	CGGTTTGGAT	AAAACAGTGT	CAGACCTGCG	CAAAGAAACC	CGCCAAGGCC
60	1001	TTGCAGAAC	AGCCGCGCTC	TCCGGTCTGT	TCCAACCTTA	CAACGTGGGT
	1051	GGATCCGGAG	GGGTGGTGT	CGCCGCCGAC	ATCGGTGCGG	GGCTTGCCGA
	1101	TGCACTAACC	GCACCGCTCG	ACCATAAAGA	CAAAGGTTTG	CAGTCTTTGA
	1151	CGCTGGATCA	GTCCGTCAGG	AAAAACGAGA	AACTGAAGCT	GGCGGCACAA
	1201	GGTGCGGAAA	AACTTATG	AAACGGTGAC	AGCCTCAATA	CGGGCAAATT
	1251	GAAGAACGAC	AAGTTCAGCC	GTTTCGACTT	TATCCGCCAA	ATCGAAGTGG
65	1301	ACGGGCAGCT	CATTACCTTG	GAGAGTGGAG	AGTTCCAAGT	ATACAAACAA
	1351	AGCCATTCCG	CCTTAACCGC	CTTTCAGACC	GAGCAAATAC	AAGATTCCGA
	1401	GCATTCCGGG	AAGATGGTTG	CGAAACGCCA	GTTTCAGAATC	GGCGACATAG

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5	1451	CGGGCGAACA	TACATCTTTT	GACAAGCTTC	CCGAAGGCGG	CAGGGCGACA
	1501	TATCGCGGGA	CGGCGTTCGG	TTCAGACGAT	GCCGGCGGAA	AACTGACCTA
	1551	CACCATAGAT	TTCGCCGCCA	AGCAGGGAAA	CGGCAAAATC	GAACATTTGA
	1601	AATCGCCAGA	ACTCAATGTC	GACCTGGCCG	CCGCCGATAT	CAAGCCGGAT
	1651	GGAAAACGCC	ATGCCGTCAT	CAGCGGTTCC	GTCTTTTACA	ACCAAGCCGA
	1701	GAAAGGCAGT	TACTCCCTCG	GTATCTTTGG	CGGAAAAGCC	CAGGAAGTTG
	1751	CCGGCAGCGC	GGAAGTGAAA	ACCGTAAACG	CACATACGCCA	TATCGGCCTT
1801	GCCGCCAAGC	AACTCGAGCA	CCACCACCAC	CACCACCTGA		
10	1	MKHFPKSVLT	TAILATFCSG	ALAATNDDDV	KKAATVAIAA	AYNNGQEING
	51	FKAGETIYDI	DEDGTITKRD	ATAADVEADD	FKGLGLKKVV	TNLTKTVNEN
	101	KQNVDAKVK	AESEIEKLTT	KLADTDAALA	DTDAALDATT	NALNKLGENI
	151	TTFAEETKTN	IVKIDEKLEA	VADTVDKHAE	AFNDIADSLD	ETNTRKAEAV
	201	KTANEAKQTA	EETKQNVDAK	VKAAETAAGK	AEAAAAGTANT	AADKAEAVAA
	251	KVTDIKADIA	TNKDNIAKKA	NSADVYTREE	SDSKFVRIDG	LNATTEKLDI
	301	RLASAEKSIA	DHDTRLNGLD	KTVSDLRKET	RQGLAEQAAL	SGLFQPYNVG
15	351	GSGGGGVAAD	IGAGLADALT	APLDHKDKGL	QSLTLDQSVR	KNEKCLKLAAQ
	401	GAEKTYGNGD	SLNTGKLNKD	KVSRDFDIFRQ	IEVDGQLITL	ESGEFQVYKQ
	451	SHSALTAFQT	EQIQDSEHSG	KMVAKRQFRI	GDIAGEHTSF	DKLPEGGRAT
	501	YRGTAFGSDD	AGGKLTYYTID	FAAKQQNGKI	EHLKSPELNV	DLAAADIKPD
	551	GKRHAVISGS	VLYNQAEKGS	YSLGIFGGKA	QEVAGSAEVK	TVNGIRHIGL
	601	AAKQLEHHHH	HH*			
25						
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55						
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	2101	ATGTATGGAG	AACCGGGTAC	AGAACCCTTT	GAGTATGGCT	CCAACCATTG
	2151	CGGAATTACT	GCCATGTGGT	GCCTGTCGGC	ACCCTATGAA	GCAAGCGTCC
	2201	GTTTCACCCG	TACAAACCCG	ATTCAAATTG	CCGGAACATC	CTTTTCCGCA
5	2251	CCCATCGTAA	CCGGCACGGC	GGCTCTGCTG	CTGCAGAAAT	ACCCGTGGAT
	2301	GAGCAACGAC	AACCTGCGTA	CCACGTTGCT	GACGACGGCT	CAGGACATCG
	2351	GTGCAGTCGG	CGTGGACAGC	AAGTTTCGGT	GGGACTGCT	GGATGCGGGT
	2401	AAGGCCATGA	ACGGACCCGC	GTCCTTTCCG	TTCGGCGACT	TTACCGCCGA
	2451	TACGAAAGGT	ACATCCGATA	TTGCCTACTC	CTTCCGTAAC	GACATTTTCTAG
10	2501	GCACGGGCGG	CCTGATCAAA	AAAGGCGGCA	GCCAACTGCA	ACTGCACGGC
	2551	AACAACACCT	ATACGGGCAA	AACCATTATC	GAAGGCGGTT	CGCTGGTGT
	2601	ATACGGCAAC	AACAAATCGG	ATATGCGCGT	CGAAACCAA	GGTGCCTGTA
	2651	TTTATAACGG	GGCGGCATCC	GGCGGCAGCC	TGAACAGCGA	CGGCATTGTC
	2701	TATCTGGCAG	ATACCGACCA	ATCCGGCGCA	AACGAAACCG	TACACATCAA
	2751	AGGCAGTCTG	CAGCTGGACG	GCAAAGGTAC	GCTGTACACA	CGTTTGGGCA
15	2801	AACTGCTGAA	AGTGGACGGT	ACGGCGATTA	TCGGCGGCAA	GCTGTACATG
	2851	TCGGCACGCG	GCAAGGGGGC	AGGCTATCTC	AACAGTACCG	GACGACGTGT
	2901	TCCCCTTCTG	AGTGGCCGCA	AAATCGGGCA	GGATTATTCT	TTCTTTCACAA
	2951	ACATCGAAAC	CGACGGCGGC	CTGCTGGCTT	CCCTCGACAG	CGTCGAAAAA
20	3001	ACAGCGGGCA	GTGAAGGCGA	CACGCTGTCC	TATTATGTCC	GTCGCGGCAA
	3051	TGCGGCACGG	ACTGCTTCGG	CAGCGGCACA	TTCCGCGCCC	GCCGGTCTGA
	3101	AACACGCCGT	AGAACAGGGC	GGCAGCAATC	TGGAACACCT	GATGGTCGAA
	3151	CTGGATGCCT	CCGAATCATC	CGCAACACCC	GAGACGGTTG	AAACTGCGGC
	3201	AGCCGACCGC	ACAGATATGC	CGGGCATCCG	CCCCTACGGC	GCAACTTTCC
25	3251	GCGCAGCGGC	AGCCGATACG	CATGCGAATG	CCGCGACGG	TGTACGCATC
	3301	TTCAACAGTC	TCGCGGCTAC	CGTCTATGCC	GACAGTACCG	CCGCCCATGC
	3351	CGATATGCAG	GGACGCCGCC	TGAAAGCCGT	ATCGGACGGG	TTGGACCACA
	3401	ACGGCACGGG	TCTGCGCGTC	ATCGCGCAAA	CCCAACAGGA	CGGTGGAACG
	3451	TGGGAACAGG	GCGGTGTTGA	AGGCAAAATG	CGCGGCAGTA	CCCAAACCGT
30	3501	CGGCATTGCC	GCGAAAACCG	GCGAAAATAC	GACAGCAGCC	GCCACACTGG
	3551	GCATGGGACG	CAGCACATGG	AGCGAAAACA	GTGCAAAATG	AAAAACCGAC
	3601	AGCATTAGTC	TGTTTGCAGG	CATACGGCAC	GATGCGGGCG	ATATCGGCTA
	3651	TCTCAAAGGC	CTGTCTCCT	ACGGACGCTA	CAAAAACAGC	ATCAGCCGCA
	3701	GCACCGGTGC	GGACGAACAT	GCGGAAGGCA	GCGTCAACGG	CACGTGATG
35	3751	CAGCTGGGCG	CACTGGGCGG	TGTCAACGTT	CCGTTTGCCG	CAACGGGAGA
	3801	TTTGACGGTC	GAAGGCGGTC	TGCGCTACGA	CCTGCTCAA	CAGGATGCAT
	3851	TCGCCGAAAA	AGGCAGTGCT	TTGGGCTGGA	GCGGCAACAG	CCTCACTGAA
	3901	GGCACGCTGG	TCGGACTCGC	GGGTCTGAAG	CTGTCGCAAC	CCTTGAGCGA
	3951	TAAAGCCGTC	CTGTTTGCAA	CGGCGGGCGT	GGAACGCGAC	CTGAACGGAC
40	4001	GCGACTACAC	GGTAACGGGC	GGCTTTACCG	GCGCGACTGC	AGCAACCGGC
	4051	AAGACGGGGG	CACGCAATAT	GCCGCACACC	CGTCTGGTTG	CCGGCCTGGG
	4101	CGCGGATGTC	GAATTCGGCA	ACGGCTGGAA	CGGCTTGCCA	CGTTACAGCT
	4151	ACGCCGGTTC	CAAACAGTAC	GGCAACCACA	GCGGACGAGT	CGGCGTAGGC
	4201	TACCGGTTCT	GACTCGAG			
45	1	MKHFPKSVLT	TAILATFCSG	ALAATNDDDV	KKAATVAIAA	AYNNGQIEING
	51	FKAGETIYDI	DEDTITTKKD	ATAADVEADD	FKGLGLKKVV	TNLTKTVNEN
	101	KQNVDAKVK	AESEIEKLTT	KLADTDAAAL	DTDAALDATT	NALNKLGENI
	151	TTFAEETKT	IVKIDKLEA	VADTVDKHAE	AFNDIADSLD	ETNTKADEAV
50	201	KTANEAKQTA	EETKQNVDAK	VKAAETAAGK	AEBAAAGTANT	AADKAEAVAA
	251	KVTDIKADIA	TNKDNIAKKA	NSADVYTREE	SDSKFVRIDG	LNATTEKLD
	301	RLASAEKSIA	DHDTRLNGLD	KTVSDLRKET	RQGLAEQAAL	SGLFQPYNVG
	351	SGSGGGTSAP	DFNAGGTGIG	SNSRATTAKS	AAVSYAGIKN	EMCKDRSMLC
	401	AGRDDVAVTD	RDakinAPPP	NLHTGDFPNP	NDAYKNLINL	KPAIEAGYTG
	451	RGVEVGIVDT	GESVGSISFP	ELYGRKEHGY	NENYKNYTAY	MRKEAPEDGG
55	501	GKDIEASFDD	EAVIETEAKP	TDIRHVKEIG	HIDLVSIIIG	GRSVDGRPAG
	551	GIAPDATHLI	MNTNDETKNE	MMVAAIRNAW	VKLGERGVRI	VNNSFGTTSR
	601	AGTADLFQIA	NSEEQYRQAL	LDYSGGDKTD	EGIRLMQSQD	YGNLSYHIRN
	651	KNMLFIFSTG	NDAQAQPNY	ALLPFYKDA	QKGIIITVAGV	DRSGEKFKE
60	701	MYGEPGTEPL	EYGSNHCGIT	AMWCLAPYE	ASVRFTRTNP	IQIAGTSFSA
	751	PIVTGTAALL	LQKYFWSND	NLRTTLLTTA	QDIGAVGVDS	KFGWGLLDAG
	801	KAMNGPASFP	FGDPTADTKG	TSDIAYSFRN	DISGTGGLIK	KGGSQQLQHLG
	851	NNTYTGTII	EGGSLVLYGN	NKSMDRVETK	GALIYNGAAS	GGSLNSDGIV
	901	YLADTDQSGA	NETVHIKGS	QLDGKGTLYT	RLGKLLKVDG	TAIIGKLYM
	951	SARGKGAGYL	NSTGRRVPFL	SAAKIGQDYS	FFTNIETDGG	LLASLDSVEK
65	1001	TAGSEDTLS	YVVRGNAAR	TASAAHSAP	AGLKHAVEQG	GSNLENLMVE
	1051	LDASESATP	ETVETAAADR	TDMPGIRPYG	ATFRAAAVQ	HANAADGVRI
	1101	FNSLAATVYA	DSTAAHADMQ	GRRLKAVSDG	LDHNGTGLRV	IAQTQQDGGT

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1151 WEQGGVEGKM RGSTQTVGIA AKTGENTTAA ATLMGRSTW SENSANAKTD
1201 SISLFAGIRH DAGDIGYLKG LFSYGRYKNS ISRSTGADEH AEGSVNGTLM
1251 QLGALGGVNV PFAATGDLTV EGGLRYDLLK QDAFAEKSA LGWSGNSLTE
1301 GTLVGLAGLK LSQPLSDKAV LFATAGVERD LNGRDYTVTG GFTGATAATG
1351 KTGARNMPHT RLVAGLGADV EFGNGWNGLA RYSYAGSKQY GNHSGRVGVG
1401 YRF*

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It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention. For instance, the use of proteins from other strains is envisaged [e.g. see WO00/66741 for polymorphic sequences for ORF4, ORF40, ORF46, 225, 235, 287, 519, 726, 919 and 953].

## EXPERIMENTAL DETAILS

### *FPLC protein purification*

The following table summarises the FPLC protein purification that was used:

Protein	PI	Column	Buffer	pH	Protocol
121.1 <sup>untagged</sup>	6.23	Mono Q	Tris	8.0	A
128.1 <sup>untagged</sup>	5.04	Mono Q	Bis-Tris propane	6.5	A
406.1L	7.75	Mono Q	Diethanolamine	9.0	B
576.1L	5.63	Mono Q	Tris	7.5	B
593 <sup>untagged</sup>	8.79	Mono S	Hepes	7.4	A
726 <sup>untagged</sup>	4.95	Hi-trap S	Bis-Tris	6.0	A
919 <sup>untagged</sup>	10.5(-leader)	Mono S	Bicine	8.5	C
919Lorf4	10.4(-leader)	Mono S	Tris	8.0	B
920L	6.92(-leader)	Mono Q	Diethanolamine	8.5	A
953L	7.56(-leader)	Mono S	MES	6.6	D
982 <sup>untagged</sup>	4.73	Mono Q	Bis-Tris propane	6.5	A
919-287	6.58	Hi-trap Q	Tris	8.0	A
953-287	4.92	Mono Q	Bis-Tris propane	6.2	A

Buffer solutions included 20-120 mM NaCl, 5.0 mg/ml CHAPS and 10% v/v glycerol. The dialysate was centrifuged at 13000g for 20 min and applied to either a mono Q or mono S FPLC ion-exchange resin. Buffer and ion exchange resins were chosen according to the pI of the protein of interest and the recommendations of the FPLC protocol manual [Pharmacia: *FPLC Ion Exchange and Chromatofocussing; Principles and Methods*. Pharmacia

Publication]. Proteins were eluted using a step-wise NaCl gradient. Purification was analysed by SDS-PAGE and protein concentration determined by the Bradford method.

The letter in the 'protocol' column refers to the following:

**FPLC-A:** Clones 121.1, 128.1, 593, 726, 982, periplasmic protein 920L and hybrid proteins  
5 919-287, 953-287 were purified from the soluble fraction of *E.coli* obtained after disruption of the cells. Single colonies harbouring the plasmid of interest were grown overnight at 37°C in 20 ml of LB/Amp (100 µg/ml) liquid culture. Bacteria were diluted 1:30 in 1.0 L of fresh medium and grown at either 30°C or 37°C until the OD<sub>550</sub> reached 0.6-0.8. Expression of recombinant protein was induced with IPTG at a final concentration of 1.0 mM. After  
10 incubation for 3 hours, bacteria were harvested by centrifugation at 8000g for 15 minutes at 4°C. When necessary cells were stored at -20°C. All subsequent procedures were performed on ice or at 4°C. For cytosolic proteins (121.1, 128.1, 593, 726 and 982) and periplasmic protein 920L, bacteria were resuspended in 25 ml of PBS containing complete protease inhibitor (Boehringer-Mannheim). Cells were lysed by sonication using a Branson  
15 Sonifier 450. Disrupted cells were centrifuged at 8000g for 30 min to sediment unbroken cells and inclusion bodies and the supernatant taken to 35% v/v saturation by the addition of 3.9 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The precipitate was sedimented at 8000g for 30 minutes. The supernatant was taken to 70% v/v saturation by the addition of 3.9 M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and the precipitate collected as above. Pellets containing the protein of interest were identified by SDS-PAGE  
20 and dialysed against the appropriate ion-exchange buffer (see below) for 6 hours or overnight. The periplasmic fraction from *E.coli* expressing 953L was prepared according to the protocol of Evans *et. al.* [*Infect.Immun.* (1974) 10:1010-1017] and dialysed against the appropriate ion-exchange buffer. Buffer and ion exchange resin were chosen according to the pI of the protein of interest and the recommendations of the FPLC protocol manual (Pharmacia). Buffer solutions included 20 mM NaCl, and 10% (v/v) glycerol. The dialysate  
25 was centrifuged at 13000g for 20 min and applied to either a mono Q or mono S FPLC ion-exchange resin. Buffer and ion exchange resin were chosen according to the pI of the protein of interest and the recommendations of the FPLC protocol manual (Pharmacia). Proteins were eluted from the ion-exchange resin using either step-wise or continuous NaCl  
30 gradients. Purification was analysed by SDS-PAGE and protein concentration determined by Bradford method. Cleavage of the leader peptide of periplasmic proteins was demonstrated by sequencing the NH<sub>2</sub>-terminus (see below).

**FPLC-B:** These proteins were purified from the membrane fraction of *E.coli*. Single colonies harbouring the plasmid of interest were grown overnight at 37°C in 20 ml of LB/Amp (100 µg/ml) liquid culture. Bacteria were diluted 1:30 in 1.0 L of fresh medium. Clones 406.1L and 919LOrf4 were grown at 30°C and Orf25L and 576.1L at 37°C until the OD<sub>550</sub> reached 0.6-0.8. In the case of 919LOrf4, growth at 30°C was essential since expression of recombinant protein at 37°C resulted in lysis of the cells. Expression of recombinant protein was induced with IPTG at a final concentration of 1.0 mM. After incubation for 3 hours, bacteria were harvested by centrifugation at 8000g for 15 minutes at 4°C. When necessary cells were stored at -20 °C. All subsequent procedures were performed at 4°C. Bacteria were resuspended in 25 ml of PBS containing complete protease inhibitor (Boehringer-Mannheim) and lysed by osmotic shock with 2-3 passages through a French Press. Unbroken cells were removed by centrifugation at 5000g for 15 min and membranes precipitated by centrifugation at 100000g (Beckman Ti50, 38000rpm) for 45 minutes. A Dounce homogenizer was used to re-suspend the membrane pellet in 7.5 ml of 20 mM Tris-HCl (pH 8.0), 1.0 M NaCl and complete protease inhibitor. The suspension was mixed for 2-4 hours, centrifuged at 100000g for 45 min and the pellet resuspended in 7.5 ml of 20mM Tris-HCl (pH 8.0), 1.0M NaCl, 5.0mg/ml CHAPS, 10% (v/v) glycerol and complete protease inhibitor. The solution was mixed overnight, centrifuged at 100000g for 45 minutes and the supernatant dialysed for 6 hours against an appropriately selected buffer. In the case of Orf25.L, the pellet obtained after CHAPS extraction was found to contain the recombinant protein. This fraction, without further purification, was used to immunise mice.

**FPLC-C:** Identical to FPLC-A, but purification was from the soluble fraction obtained after permeabilising *E.coli* with polymyxin B, rather than after cell disruption.

**FPLC-D:** A single colony harbouring the plasmid of interest was grown overnight at 37°C in 20 ml of LB/Amp (100 µg/ml) liquid culture. Bacteria were diluted 1:30 in 1.0 L of fresh medium and grown at 30°C until the OD<sub>550</sub> reached 0.6-0.8. Expression of recombinant protein was induced with IPTG at a final concentration of 1.0mM. After incubation for 3 hours, bacteria were harvested by centrifugation at 8000g for 15 minutes at 4°C. When necessary cells were stored at -20 °C. All subsequent procedures were performed on ice or at 4°C. Cells were resuspended in 20mM Bicine (pH 8.5), 20mM NaCl, 10% (v/v) glycerol, complete protease inhibitor (Boehringer-Mannheim) and disrupted using a Branson Sonifier 450. The sonicate was centrifuged at 8000g for 30 min to sediment unbroken cells and

inclusion bodies. The recombinant protein was precipitated from solution between 35% v/v and 70% v/v saturation by the addition of 3.9M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. The precipitate was sedimented at 8000g for 30 minutes, resuspended in 20 mM Bicine (pH 8.5), 20 mM NaCl, 10% (v/v) glycerol and dialysed against this buffer for 6 hours or overnight. The dialysate was  
 5 centrifuged at 13000g for 20 min and applied to the FPLC resin. The protein was eluted from the column using a step-wise NaCl gradients. Purification was analysed by SDS-PAGE and protein concentration determined by Bradford method.

### *Cloning strategy and oligonucleotide design*

Genes coding for antigens of interest were amplified by PCR, using oligonucleotides  
 10 designed on the basis of the genomic sequence of *N. meningitidis* B MC58. Genomic DNA from strain 2996 was always used as a template in PCR reactions, unless otherwise specified, and the amplified fragments were cloned in the expression vector pET21b+ (Novagen) to express the protein as C-terminal His-tagged product, or in pET-24b+(Novagen) to express the protein in 'untagged' form (*e.g.* ΔG 287K).

15 Where a protein was expressed without a fusion partner and with its own leader peptide (if present), amplification of the open reading frame (ATG to STOP codons) was performed.\*

Where a protein was expressed in 'untagged' form, the leader peptide was omitted by designing the 5'-end amplification primer downstream from the predicted leader sequence.

The melting temperature of the primers used in PCR depended on the number and type of  
 20 hybridising nucleotides in the whole primer, and was determined using the formulae:

$$T_{m1} = 4 (G+C) + 2 (A+T) \quad \text{(tail excluded)}$$

$$T_{m2} = 64.9 + 0.41 (\% \text{ GC}) - 600/N \quad \text{(whole primer)}$$

The melting temperatures of the selected oligonucleotides were usually 65-70°C for the whole oligo and 50-60°C for the hybridising region alone.

25 Oligonucleotides were synthesised using a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2.0ml NH<sub>4</sub>OH, and deprotected by 5 hours incubation at 56°C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were centrifuged and the pellets resuspended in water.

		Sequences	Restriction site
Orf1L	Fwd	CGCGGATCCGCTAGC-AAAACAACCGACAAACGG	NheI
	Rev	CCCGCTCGAG-TTACCAGCGGTAGCCTA	XhoI
Orf1	Fwd	CTAGCTAGC-GGACACACTTATTTTCGGCATC	NheI
	Rev	CCCGCTCGAG-TTACCAGCGGTAGCCTAATTTG	XhoI
Orf1LOmpA	Fwd		NdeI-(NheI)
	Rev	CCCGCTCGAG-	XhoI
Orf4L	Fwd	CGCGGATCCCATATG-AAAACCTTCTTCAAAACC	NdeI
	Rev	CCCGCTCGAG-TTATTTGGCTGCGCCTTC	XhoI
Orf7-1L	Fwd	GCGGCATTAAT-ATGTTGAGAAAATTGTTGAAATGG	AseI
	Rev	GCGGCCTCGAG-TTATTTTTTCAAAATATATTTGC	XhoI
Orf9-1L	Fwd	GCGGCCATATG-TTACCTAACCGTTTCAAAATGT	NdeI
	Rev	GCGGCCTCGAG-TTATTTCCGAGGTTTTTCGGG	XhoI
Orf23L	Fwd	CGCGGATCCCATATG-ACACGCTTCAAATATTC	NdeI
	Rev	CCCGCTCGAG-TTATTTAAACCGATAGGTAAA	XhoI
Orf25-1 His	Fwd	CGCGGATCCCATATG-GGCAGGGAAGAACCGC	NdeI
	Rev	GCCCAAGCTT-ATCGATGGAATAGCCGCG	HindIII
Orf29-1 b-His (MC58)	Fwd	CGCGGATCCGCTAGC-AACGTTTGGATGCCCCG	NheI
	Rev	CCCGCTCGAG-TTTGTCTAAGTTCCTGATAT CCCGCTCGAG-ATTCCACCTGCCATC	XhoI
Orf29-1 b-L (MC58)	Fwd	CGCGGATCCGCTAGC-ATGAATTTGCCTATTCAAAAAT	NheI
	Rev	CCCGCTCGAG-TTAATTTCCACCTGCCATC	XhoI
Orf29-1 c-His (MC58)	Fwd	CGCGGATCCGCTAGC-ATGAATTTGCCTATTCAAAAAT	NheI
	Rev	CCCGCTCGAG-TTGGACGATGCCCCGGA	XhoI
Orf29-1 c-L (MC58)	Fwd	CGCGGATCCGCTAGC-ATGAATTTGCCTATTCAAAAAT	NheI
	Rev	CCCGCTCGAG-TTATTGGACGATGCCCCG	XhoI
Orf25L	Fwd	CGCGGATCCCATATG-TATCGCAAACGATTGC	NdeI
	Rev	CCCGCTCGAG-CTAATCGATGGAATAGCC	XhoI
Orf37L	Fwd	CGCGGATCCCATATG-AAACAGACAGTCAAATG	NdeI
	Rev	CCCGCTCGAG-TCAATAACCCGCCTTCAG	XhoI
Orf38L	Fwd	CGCGGATCCCATATG- TTACGTTTGACTGCTTTAGCCGTATGCACC	NdeI
	Rev	CCCGCTCGAG- TTATTTTGCCGCGTTAAAGCGTCGGCAAC	XhoI
Orf40L	Fwd	CGCGGATCCCATATG-AACAAAATATACCGCAT	NdeI
	Rev	CCCGCTCGAG-TTACCACTGATAACCGAC	XhoI
Orf40.2-His	Fwd	CGCGGATCCCATATG-ACCGATGACGACGATTTAT	NdeI
	Rev	GCCCAAGCTT-CCACTGATAACCGACAGA	HindIII
Orf40.2L	Fwd	CGCGGATCCCATATG-AACAAAATATACCGCAT	NdeI
	Rev	GCCCAAGCTT-TTACCACTGATAACCGAC	HindIII
Orf46-2L	Fwd	GGAATTCATATG-GGCATTTCCCGCAAAATATC	NdeI
	Rev	CCCGCTCGAG-TTATTTACTCCTATAACGAGGTCTCTTAAC	XhoI
Orf46-2	Fwd	GGAATTCATATG-TCAGATTTGGCAAACGATTCTT	NdeI
	Rev	CCCGCTCGAG-TTATTTACTCCTATAACGAGGTCTCTTAAC	XhoI
Orf46.1L	Fwd	GGAATTCATATG-GGCATTTCCCGCAAAATATC	NdeI

	Rev	CCCGCTCGAG-TTACGTATCATATTTACGTGC	XhoI
orf46. (His-GST)	Fwd	GGGAATTCCATATGCACGTGAAATATGATACGAAG	BamHI-NdeI
	Rev	CCCGCTCGAGTTTACTCCTATAACGAGGTCTCTTAAC	XhoI
orf46.1-His	Fwd	GGGAATTCCATATGTCAGATTTGGCAAACGATTCTT	NdeI
	Rev	CCCGCTCGAGCGTATCATATTTACGTGC	XhoI
orf46.2-His	Fwd	GGGAATTCCATATGTCAGATTTGGCAAACGATTCTT	NdeI
	Rev	CCCGCTCGAGTTTACTCCTATAACGAGGTCTCTTAAC	XhoI
Orf65-1-(His/GST) (MC58)	Fwd	CGCGGATCCCATATG-CAAAATGCGTTCAAAATCCC	BamHI-NdeI
	Rev	CGCGGATCCCATATG-AACAAAATATACCGCAT CCCGCTCGAG-TTTGCTTCGATAGAACGG	XhoI
Orf72-1L	Fwd	GCGGCCATATG-GTCATAAAATATACAAATTTGAA	NdeI
	Rev	GCGGCCTCGAG-TTAGCCTGAGACCTTTGCAAATT	XhoI
Orf76-1L	Fwd	GCGGCCATATG-AAACAGAAAAAACCCTG	NdeI
	Rev	GCGGCCTCGAG-TTACGGTTTGACACCGTTTTTC	XhoI
Orf83.1L	Fwd	CGCGGATCCCATATG-AAAACCTGCTCCTC	NdeI
	Rev	CCCGCTCGAG-TTATCCTCCTTTGCGGC	XhoI
Orf85-2L	Fwd	GCGGCCATATG-GCAAAAATGATGAAATGGG	NdeI
	Rev	GCGGCCTCGAG-TTATCGGCGCGGCGGGCC	XhoI
Orf91L (MC58)	Fwd	GCGGCCATATGAAAAAATCCTCCCTCATCA	NdeI
	Rev	GCGGCCTCGAGTTATTTGCCGCCGTTTTTGGC	XhoI
Orf91-His(MC58)	Fwd	GCGGCCATATGGCCCCTGCCGACGCGGTAAG	NdeI
	Rev	GCGGCCTCGAGTTTGCCGCCGTTTTTGGCTTTC	XhoI
Orf97-1L	Fwd	GCGGCCATATG-AAACACATACTCCCCCTGA	NdeI
	Rev	GCGGCCTCGAG-TTATTGCCTACGGTTTTTTG	XhoI
Orf119L (MC58)	Fwd	GCGGCCATATGATTTACATCGTACTGTTTC	NdeI
	Rev	GCGGCCTCGAGTTAGGAGAACAGGCGCAATGC	XhoI
Orf119-His(MC58)	Fwd	GCGGCCATATGTACAACATGTATCAGGAAAAC	NdeI
	Rev	GCGGCCTCGAGGAGAACAGGCGCAATGCGG	XhoI
Orf137.1 (His-GST) (MC58)	Fwd	CGCGGATCCGCTAGCTGCGGCACGGCGGG	BamHI-NheI
	Rec	CCCGCTCGAGATAACGGTATGCCGCCAG	XhoI
Orf143-1L	Fwd	CGCGGATCCCATATG-GAATCAACACTTTCAC	NdeI
	Rev	CCCGCTCGAG-TTACACGCGGTTGCTGT	XhoI
008	Fwd	CGCGGATCCCATATG-AACAACAGACATTTTG	NdeI
	Rev	CCCGCTCGAG-TTACCTGTCCGGTAAAAG	XhoI
050-1(48)	Fwd	CGCGGATCCGCTAGC-ACCGTCATCAAACAGGAA	NheI
	Rev	CCCGCTCGAG-TCAAGATTCGACGGGGA	XhoI
105	Fwd	CGCGGATCCCATATG-TCCGCAAACGAATACG	NdeI
	Rev	CCCGCTCGAG-TCAGTGTTCTGCCAGTTT	XhoI
111L	Fwd	CGCGGATCCCATATG-CCGTCTGAAACACG	NdeI
	Rev	CCCGCTCGAG-TTAGCGGAGCAGTTTTTC	XhoI
117-1	Fwd	CGCGGATCCCATATG-ACCGCCATCAGCC	NdeI
	Rev	CCCGCTCGAG-TTAAAGCCGGGTAACGC	XhoI
121-1	Fwd	GCGGCCATATG-GAAACACAGCTTTACATCGG	NdeI
	Rev	GCGGCCTCGAG-TCAATAATAATATCCCGCG	XhoI

122-1	Fwd	GCGGCCATATG-ATTAAATCCGCAATATCC	NdeI
	Rev	GCGGCCTCGAG-TTAAATCTTGGTAGATTGGATTGG	XhoI
128-1	Fwd	GCGGCCATATG-ACTGACAACGCACTGCTCC	NdeI
	Rev	GCGGCCTCGAG-TCAGACCGCGTTGTCGAAAC	XhoI
148	Fwd	CGCGGATCCCATATG-GCGTTAAAAACATCAAA	NdeI
	Rev	CCCGCTCGAG-TCAGCCCTTCATACAGC	XhoI
149.1L (MC58)	Fwd	GCGGCATTAATGGCACAACACTACACTCAAACC	AseI
	Rev	GCGGCCTCGAGTTAAAACTTCACGTTACGCCC	XhoI
149.1-His(MC58)	Fwd	GCGGCATTAATGCATGAAACTGAGCAATCGGTGG	AseI
	Rev	GCGGCCTCGAGAAACTTCACGTTACGCGCCCGGTAA	XhoI
205 (His-GST) (MC58)	Fwd	CGCGGATCCCATATGGGCAAATCCGAAAATACG	BamHI-NdeI
	Rev	CCCGCTCGAGATAATGGCGGCGGCGG	XhoI
206L	Fwd	CGCGGATCCCATATG-TTCCCCCGACAA	NdeI
	Rev	CCCGCTCGAG-TCATTCTGTAAAAAAGTATG	XhoI
214 (His-GST) (MC58)	Fwd	CGCGGATCCCATATGCTTCAAAGCGACAGCAG	BamHI-NdeI
	Rev	CCCGCTCGAGTTCGGATTTTTCGTACTC	XhoI
216	Fwd	CGCGGATCCCATATG-GCAATGGCAGAAAACG	NdeI
	Rev	CCCGCTCGAG-CTATACAATCCGTGCCG	XhoI
225-1L	Fwd	CGCGGATCCCATATG-GATTCTTTTTTCAAACC	NdeI
	Rev	CCCGCTCGAG-TCAGTTCAGAAAGCGGG	XhoI
235L	Fwd	CGCGGATCCCATATG-AAACCTTTGATTTTAGG	NdeI
	Rev	CCCGCTCGAG-TTATTTGGGCTGCTCTTC	XhoI
243	Fwd	CGCGGATCCCATATG-GTAATCGTCTGGTTG	NdeI
	Rev	CCCGCTCGAG-CTACGACTTGGTTACCG	XhoI
247-1L	Fwd	GCGGCCATATG-AGACGTAAAATGCTAAAGCTAC	NdeI
	Rev	GCGGCCTCGAG-TCAAAGTGTTCTGTTGCGC	XhoI
264-His	Fwd	GCCGCCATATG-TTGACTTTAACCCGAAAAA	NdeI
	Rev	GCCGCCTCGAG-GCCGGCGGTCAATACCGCCCGAA	XhoI
270 (His-GST) (MC58)	Fwd	CGCGGATCCCATATGGCGCAATGCGATTGAC	BamHI-NdeI
	Rev	CCCGCTCGAGTTCGGCGGTAAATGCCG	XhoI
274L	Fwd	GCGGCCATATG-GCGGGCCGATTTTTGT	NdeI
	Rev	GCGGCCTCGAG-TTATTTGCTTTTCAGTATTATTG	XhoI
283L	Fwd	GCGGCCATATG-AACTTTGCTTTATCCGTCA	NdeI
	Rev	GCGGCCTCGAG-TTAACGGCAGTATTGTTTAC	XhoI
285-His	Fwd	CGCGGATCCCATATGGGTTTTCGCTTCGGGC	BamHI
	Rev	GCCCAAGCTTTTTCCTTTGCCGTTCCG	HindIII
286-His (MC58)	Fwd	CGCGGATCCCATATG-GCCGACCTTCCGAAAA	NdeI
	Rev	CCCGCTCGAG-GAAGCGCGTTCCCAAGC	XhoI
286L (MC58)	Fwd	CGCGGATCCCATATG-CACGACACCCGTAC	NdeI
	Rev	CCCGCTCGAG-TTAGAAGCGCGTTCCCAA	XhoI
287L	Fwd	CTAGCTAGC-TTTAAACGCAGCGTAATCGCAATGG	NheI
	Rev	CCCGCTCGAG-TCAATCCTGCTCTTTTTTGCC	XhoI

287	Fwd	CTAGCTAGC-GGGGGCGGCGGTGGCG	NheI
	Rev	CCCCTCGAG-TCAATCTGCTCTTTTTTGCC	XhoI
287Lorf4	Fwd	CTAGCTAGCGCTCATCTCGCCGCC-TGCGGGGGCGGCGGT	NheI
	Rev	CCCCTCGAG-TCAATCTGCTCTTTTTTGCC	XhoI
287-fu	Fwd	CGGGGATCC-GGGGGCGGCGGTGGCG	BamHI
	Rev	CCCCTCGAG-TCAATCTGCTCTTTTTTGCC	XhoI
287-His	Fwd	CTAGCTAGC-GGGGGCGGCGGTGGCG	NheI
	Rev	CCCCTCGAG-ATCCTGCTCTTTTTTGCC *	XhoI
287-His(2996)	Fwd	CTAGCTAGC-TGCGGGGGCGGCGGTGGCG	NheI
	Rev	CCCCTCGAG-ATCCTGCTCTTTTTTGCC	XhoI
Δ1 287-His	Fwd	CGCGGATCCGCTAGC-CCCGATGTAAATCGGC <sup>§</sup>	NheI
Δ2 287-His	Fwd	CGCGGATCCGCTAGC-CAAGATATGGCGGAGT <sup>§</sup>	NheI
Δ3 287-His	Fwd	CGCGGATCCGCTAGC-GCCGAATCCGCAAATCA <sup>§</sup>	NheI
Δ4 287-His	Fwd	CGCGCTAGC-GGAAGGGTTGATTGGCTAATGG <sup>§</sup>	NheI
Δ4 287MC58-His	Fwd	CGCGCTAGC-GGAAGGGTTGATTGGCTAATGG <sup>§</sup>	NheI
287a-His	Fwd	CGCCATATG-TTTAAACGCAGCGTAATCGC	NdeI
	Rev	CCCCTCGAG-AAAATTGCTACCGCCATTCGCAGG	XhoI
287b-His	Fwd	CGCCATATG-GGAAGGGTTGATTGGCTAATGG	NdeI
287b-2996-His	Rev	CCCCTCGAG-CTTGTCTTTATAAATGATGACATATTG	XhoI
287b-MC58-His	Rev	CCCCTCGAG-TTTATAAAAGATAATATATTGATTGATTCC	XhoI
287c-2996-His	Fwd	CGCGCTAGC-ATGCCGCTGATTCCCGTCAATC <sup>§</sup>	NheI
'287 <sup>untagged</sup> '(2996)	Fwd	CTAGCTAGC-GGGGGCGGCGGTGGCG	NheI
	Rev	CCCCTCGAG-TCAATCTGCTCTTTTTTGCC	XhoI
ΔG287-His *	Fwd	CGCGGATCCGCTAGC-CCCGATGTAAATCGGC	NheI
	Rev	CCCCTCGAG-ATCCTGCTCTTTTTTGCC	XhoI
ΔG287K(2996)	Fwd	CGCGGATCCGCTAGC-CCCGATGTAAATCGGC	NheI
	Rev	CCCCTCGAG-TCAATCTGCTCTTTTTTGCC	XhoI
ΔG 287-L	Fwd	CGCGGATCCGCTAGC-TTTGAACGCAGTGTGATTGCAATGGCTTGTATTTTGCCCTTTCAGCCTGT TCGCCCGATGTAAATCGGCG	NheI
	Rev	CCCCTCGAG-TCAATCTGCTCTTTTTTGCC	XhoI
ΔG 287-Orf4L	Fwd	CGCGGATCCGCTAGC-AAAACCTTCTTCAAAACCTTTCCGCCGCCGCACTCGCGCTCATCTCGCCGCCTGC TCGCCCGATGTAAATCG	NheI
	Rev	CCCCTCGAG-TCAATCTGCTCTTTTTTGCC	XhoI
292L	Fwd	CGCGGATCCCATATG-AAAACCAAGTTAATCAAA	NdeI
	Rev	CCCCTCGAG-TTATTGATTTTTCGGATGA	XhoI
308-1	Fwd	CGCGGATCCCATATG-TTAAATCGGGTATTTTATC	NdeI
	Rev	CCCCTCGAG-TTAATCCGCCATTCCCTG	XhoI
401L	Fwd	GCGGCCATATG-AAATTACAACAATTGGCTG	NdeI
	Rev	GCGGCCCTCGAG-TTACCTTACGTTTTTCAAAG	XhoI
406L	Fwd	CGCGGATCCCATATG-CAAGCACGGCTGCT	NdeI
	Rev	CCCCTCGAG-TCAAGGTTGTCCTTGTCTA	XhoI
502-1L	Fwd	CGCGGATCCCATATG-ATGAAACCGCACAAAC	NdeI
	Rev	CCCCTCGAG-TCAGTTGCTCAACACGTC	XhoI

502-A (His-GST)	Fwd	CGCGGATCCCATATGGTAGACGCGCTTAAGCA	BamHI-NdeI
	Rev	CCCGCTCGAGAGCTGCATGGCGGCG	XhoI
503-1L	Fwd	CGCGGATCCCATATG-GCACGGTCGTTATAC	NdeI
	Rev	CCCGCTCGAG-CTACCGCGCATTCTCTG	XhoI
519-1L	Fwd	GCGGCCATATG-GAATTTTTCATTATCTTGTT	NdeI
	Rev	GCGGCCTCGAG-TTATTTGGCGGTTTTGCTGC	XhoI
525-1L	Fwd	GCGGCCATATG-AAGTATGTCCGGTTATTTTC	NdeI
	Rev	GCGGCCTCGAG-TTATCGGCTTGCAACGG	XhoI
529-(His/GST) (MC58)	Fwd	CGCGGATCCGCTAGC-TCCGGCAGCAAAACCGA	Bam HI-NheI
	Rev	GCCCAAGCTT-ACGCAGTTCGGAATGGAG	HindIII
552L	Fwd	GCCGCCATATGTTGAATATTAAACTGAAAACCTTG	NdeI
	Rev	GCCGCCTCGAGTTATTTCTGATGCCTTTTCCC	XhoI
556L	Fwd	GCCGCCATATGGACAATAAGACCAAACTG	NdeI
	Rev	GCCGCCTCGAGTTAACGGTGCGGACGTTTC	XhoI
557L	Fwd	CGCGGATCCCATATG-AACAACTGTTTCTTAC	NdeI
	Rev	CCCGCTCGAG-TCATTCCGCCTTCAGAAA	XhoI
564ab-(His/GST) (MC58)	Fwd	CGCGGATCCCATATG- CAAGGTATCGTTGCCGACAAATCCGCACCT	BamHI-NdeI
	Rev	CCCGCTCGAG- AGCTAATTGTGCTTGGTTTGCAGATAGGAGTT	XhoI
564abL (MC58)	Fwd	CGCGGATCCCATATG- AACCGCACCTGTACAAAGTTGTATTTAACAAACATC	NdeI
	Rev	CCCGCTCGAG- TTAAGCTAATTGTGCTTGGTTTGCAGATAGGAGTT	XhoI
564b- (His/GST)(MC58)	Fwd	CGCGGATCCCATATG- ACGGGAGAAAATCATGCGGTTTCACTTCATG	BamHI-NdeI
	Rev	CCCGCTCGAG- AGCTAATTGTGCTTGGTTTGCAGATAGGAGTT	XhoI
564c- (His/GST)(MC58)	Fwd	CGCGGATCCCATATG- GTTTCAGACGGCCTATACAACCAACATGGTGAAATT	BamHI-NdeI
	Rev	CCCGCTCGAG- GCGGTAAGTCCGCTTGCACTGAATCCGTAA	XhoI
564bc- (His/GST)(MC58)	Fwd	CGCGGATCCCATATG- ACGGGAGAAAATCATGCGGTTTCACTTCATG	BamHI-NdeI
	Rev	CCCGCTCGAG- GCGGTAAGTCCGCTTGCACTGAATCCGTAA	XhoI
564d- (His/GST)(MC58)	Fwd	CGCGGATCCCATATG- CAAAGCAAAGTCAAAGCAGACCATGCCTCCGTAA	BamHI-NdeI
	Rev	CCCGCTCGAG- TCTTTTCCTTTCAATTATAACTTTAGTAGGTTCAATTTTG GTCCCC	XhoI
564cd- (His/GST)(MC58)	Fwd	CGCGGATCCCATATG- GTTTCAGACGGCCTATACAACCAACATGGTGAAATT	BamHI-NdeI
	Rev	CCCGCTCGAG- TCTTTTCCTTTCAATTATAACTTTAGTAGGTTCAATTTTG GTCCCC	XhoI
570L	Fwd	GCGGCCATATG-ACCCGTTTGACCCGCG	NdeI
	Rev	GCGGCCTCGAG-TCAGCGGGCGTTCATTTCTT	XhoI
576-1L	Fwd	CGCGGATCCCATATG-AACACCATTTTCAAAATC	NdeI
	Rev	CCCGCTCGAG-TTAATTTACTTTTTTGATGTCC	XhoI

580L	Fwd	GCGGCCATATG-GATTGCCCCAAGGTCGG	NdeI
	Rev	GCGGCCTCGAG-CTACACTTCCCCCGAAGTGG	XhoI
583L	Fwd	CGCGGATCCCATATG-ATAGTTGACCAAAGCC	NdeI
	Rev	CCCGCTCGAG-TTATTTTCCGATTTTTCGG	XhoI
593	Fwd	GCGGCCATATG-CTTGAACCTGAACGGACT	NdeI
	Rev	GCGGCCTCGAG-TCAGCGGAAGCGGACGATT	XhoI
650 (His-GST) (MC58)	Fwd	CGCGGATCCCATATGTCCAAACTCAAAACCATCG	BamHI-NdeI
	Rev	CCCGCTCGAGGCTTCCAATCAGTTTGACC	XhoI
652	Fwd	GCGGCCATATG-AGCGCAATCGTTGATATTTTC	NdeI
	Rev	GCGGCCTCGAG-TTATTTGCCAGTTGGTAGAATG	XhoI
664L	Fwd	GCGGCCATATG-GTGATACATCCGCACTACTTC	NdeI
	Rev	GCGGCCTCGAG-TCAAAATCGAGTTTTACACCA	XhoI
726	Fwd	GCGGCCATATG-ACCATCTATTTCAAAAACGG	NdeI
	Rev	GCGGCCTCGAG-TCAGCCGATGTTTAGCGTCCATT	XhoI
741-His(MC58)	Fwd	CGCGGATCCCATATG-AGCAGCGGAGGGGGTG	NdeI
	Rev	CCCGCTCGAG-TTGCTTGCGGCAAGGC	XhoI
ΔG741-His(MC58)	Fwd	CGCGGATCCCATATG-GTCGCCGCCGACATCG	NdeI
	Rev	CCCGCTCGAG-TTGCTTGCGGCAAGGC	XhoI
686-2-(His/GST) (MC58)	Fwd	CGCGGATCCCATATG-GGCGGTTGGAAGGCG	BamHI-NdeI
	Rev	CCCGCTCGAG-TTGAACACTGATGTCTTTTCCGA	XhoI
719-(His/GST) (MC58)	Fwd	CGCGGATCCGCTAGC-AAACTGTCGTTGGTGTTAAC	BamHI-NheI
	Rev	CCCGCTCGAG-TTGACCCGCTCCACGG	XhoI
730-His (MC58)	Fwd	GCCGCCATATGGCGGACTTGCGCAAGACCC	NdeI
	Rev	GCCGCCTCGAGATCTCCTAAACCTGTTTTAACAATGCCG	XhoI
730A-His (MC58)	Fwd	GCCGCCATATGGCGGACTTGCGCAAGACCC	NdeI
	Rev	GCGGCCTCGAGCTCCATGCTGTTGCCCCAGC	XhoI
730B-His (MC58)	Fwd	GCCGCCATATGGCGGACTTGCGCAAGACCC	NdeI
	Rev	GCGGCCTCGAGAAAATCCCCGCTAACCGCAG	XhoI
741-His (MC58)	Fwd	CGCGGATCCCATATG-AGCAGCGGAGGGGGTG	NdeI
	Rev	CCCGCTCGAG-TTGCTTGCGGCAAGGC	XhoI
ΔG741-His (MC58)	Fwd	CGCGGATCCCATATG-GTCGCCGCCGACATCG	NdeI
	Rev	CCCGCTCGAG-TTGCTTGCGGCAAGGC	XhoI
743 (His-GST)	Fwd	CGCGGATCCCATATGGACGGTGTGTGCCTGTT	BamHI-NdeI
	Rev	CCCGCTCGAGCTTACGGATCAAATTGACG	XhoI
757 (His-GST) (MC58)	Fwd	CGCGGATCCCATATGGGCAGCCAATCTGAAGAA	BamHI-NdeI
	Rev	CCCGCTCGAGCTCAGCTTTTGCCGTCAA	XhoI
759-His/GST (MC58)	Fwd	CGCGGATCCGCTAGC-TACTCATCCATTGTCCGC	BamHI-NheI
	Rev	CCCGCTCGAG-CCAGTTGTAGCCTATTTG	XhoI
759L (MC58)	Fwd	CGCGGATCCGCTAGC-ATGCGCTTACACACAC	NheI
	Rev	CCCGCTCGAG-TTACCAGTTGTAGCCTATTT	XhoI
760-His	Fwd	GCCGCCATATGGCACAAACGGAAGGTTTGAA	NdeI
	Rev	GCCGCCTCGAGAAAACCTGTAACGCAGGTTTGCCGTC	XhoI
769-His (MC58)	Fwd	GCGGCCATATGGAAGAAACACCGCGCAACCG	NdeI

	Rev	GCGGCCTCGAGGAACGTTTTATTAACTCGAC	XhoI
907L	Fwd	GCGGCCATATG-AGAAAACCGACCGATACCCTA	NdeI
	Rev	GCGGCCTCGAG-TCAACGCCACTGCCAGCGGTTG	XhoI
911L	Fwd	CGCGGATCCCATATG-AAGAAGAACATATTGGAATTTGGGTCGGACTG	NdeI
	Rev	CCCGCTCGAG-TTATTCGGCGGCTTTTTCCGCATTGCCG	XhoI
911LOmpA	Fwd	GGGAATTCCATATGAAAAAGACAGCTATCGCGATTGCA GTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCCGC TAGC-GCTTTCCGCGTGGCCGGCGGTGC	NdeI-(NheI)
	Rev	CCCGCTCGAG-TTATTCGGCGGCTTTTTCCGCATTGCCG	XhoI
911LPelB	Fwd	CATGCCATGG-CTTTCGCGTGGCCGGCGGTGC	NcoI
	Rev	CCCGCTCGAG-TTATTCGGCGGCTTTTTCCGCATTGCCG	XhoI
913-His/GST (MC58)	Fwd	CGCGGATCCCATATG-TTGCCGAAACCCGCC	BamHI-NdeI
	Rev	CCCGCTCGAG-AGGTTGTGTTCCAGGTTG	XhoI
913L (MC58)	Fwd	CGCGGATCCCATATG-AAAAAAACCGCCTATG	NdeI
	Rev	CCCGCTCGAG-TTAAGGTTGTGTTCCAGG	XhoI
919L	Fwd	CGCGGATCCCATATG-AAAAAATACCTATTCCGC	NdeI
	Rev	CCCGCTCGAG-TTACGGGCGGTATTCGG	XhoI
919	Fwd	CGCGGATCCCATATG-CAAAGCAAGAGCATCCAAA	NdeI
	Rev	CCCGCTCGAG-TTACGGGCGGTATTCGG	XhoI
919L Orf4	Fwd	GGGAATTCCATATGAAAACCTTCTTCAAAACCTTTCCG CCGCCGCTAGCGCTATCCTCGCCGCC- TGCCAAAGCAAGAGCATC	NdeI-(NheI)
	Rev	CCCGCTCGAG-TTACGGGCGGTATTCGGGCTTCATACCG	XhoI
(919)-287fusion	Fwd	CGCGGATCCGTCGAC-TGTGGGGGCGGCGGTGGC	SalI
	Rev	CCCGCTCGAG-TCAATCTGCTCTTTTTTGCC	XhoI
920-1L	Fwd	GCGGCCATATG-AAGAAAACATTGACACTGC	NdeI
	Rev	GCGGCCTCGAG-TTAATGGTGCGAATGACCGAT	XhoI
925-His/GST (MC58) <sup>GATE</sup>	Fwd	ggggacaagttgtacaaaaagcaggctTGCGGCAAGGATGCCGG	attB1
	Rev	ggggaccactttgtacaagaagctgggtCTAAAGCAACAATGCCGG	attB2
926L	Fwd	CGCGGATCCCATATG-AAACACACCGTATCC	NdeI
	Rev	CCCGCTCGAG-TTATCTCGTGC GCGCC	XhoI
927-2-(His/GST) (MC58)	Fwd	CGCGGATCCCATATG-AGCCCCGCGCCGATT	BamHI-NdeI
	Rev	CCCGCTCGAG-TTTTTGTGCGGTCAGGCG	XhoI
932-His/GST (MC58) <sup>GATE</sup>	Fwd	ggggacaagttgtacaaaaagcaggctTGTTCTGTTTGGGGGATTTAA ACCAAACCAAATC	attB1
935 (His-GST) (MC58)	For	CGCGGATCCCATATGGCGGATGCGCCCGCG	BamHI-NdeI
	Rev	CCCGCTCGAGAAACCGCCAATCCGCC	XhoI
936-1L	Rev	ggggaccactttgtacaagaagctgggtTCATTTTGTTCCTTCTTCT CGAGGCCATT	attB2
	Fwd	CGCGGATCCCATATG-AAACCCAAACCGCAC	NdeI
	Rev	CCCGCTCGAG-TCAGCGTTGGACGTAGT	XhoI
953L	Fwd	GGGAATTCCATATG-AAAAAATCATCTTCGCCG	NdeI
	Rev	CCCGCTCGAG-TTATTGTTTGGCTGCCTCGAT	XhoI
953-fu	Fwd	GGGAATTCCATATG-GCCACCTACAAAGTGGACG	NdeI
	Rev	CGGGGATCC-TTGTTTGGCTGCCTCGATTG	BamHI

954 (His-GST) (MC58)	Fwd	CGCGGATCCCATATGCAAGAACAATCGCAGAAAG	BamHI-NdeI
	Rev	CCCGCTCGAGTTTTTCGGCAAATTGGCTT	XhoI
958-His/GST (MC58) <sup>GATE</sup>	Fwd	ggggacaagtgtgtacaaaaagcaggctGCCGATGCCGTTGCCG	attB1
	Rev	ggggaccactttgtacaagaagctgggtTCAGGGTCGTTTGTGCG	attB2
961L	Fwd	CGCGGATCCCATATG-AAACACTTTCCATCC	NdeI
	Rev	CCCGCTCGAG-TTACCACTCGTAATTGAC	XhoI
961	Fwd	CGCGGATCCCATATG-GCCACAAGCGACGAC	NdeI
	Rev	CCCGCTCGAG-TTACCACTCGTAATTGAC	XhoI
961 c (His/GST)	Fwd	CGCGGATCCCATATG-GCCACAAACGACG	BamHI-NdeI
	Rev	CCCGCTCGAG-ACCCACGTTGTAAGGTTG	XhoI
961 c-(His/GST) (MC58)	Fwd	CGCGGATCCCATATG-GCCACAAGCGACGACGA	BamHI-NdeI
	Rev	CCCGCTCGAG-ACCCACGTTGTAAGGTTG	XhoI
961 c-L	Fwd	CGCGGATCCCATATG-ATGAAACACTTTCCATCC	NdeI
	Rev	CCCGCTCGAG-TTAACCCACGTTGTAAGGT	XhoI
961 c-L (MC58)	Fwd	CGCGGATCCCATATG-ATGAAACACTTTCCATCC	NdeI
	Rev	CCCGCTCGAG-TTAACCCACGTTGTAAGGT	XhoI
961 d (His/GST)	Fwd	CGCGGATCCCATATG-GCCACAAACGACG	BamHI-NdeI
	Rev	CCCGCTCGAG-GTCTGACACTGTTTTATCC	XhoI
961 Δ1-L	Fwd	CGCGGATCCCATATG-ATGAAACACTTTCCATCC	NdeI
	Rev	CCCGCTCGAG-TTATGCTTTGGCGGCAAAG	XhoI
fu 961-...	Fwd	CGCGGATCCCATATG- GCCACAAACGACGAC	NdeI
	Rev	CGCGGATCC-CCACTCGTAATTGACGCC	BamHI
fu 961-... (MC58)	Fwd	CGCGGATCCCATATG-GCCACAAGCGACGAC	NdeI
	Rev	CGCGGATCC-CCACTCGTAATTGACGCC	BamHI
fu 961 c -...	Fwd	CGCGGATCCCATATG-GCCACAAACGACGAC	NdeI
	Rev	CGCGGATCC -ACCCACGTTGTAAGGTTG	BamHI
fu 961 c-L-...	Fwd	CGCGGATCCCATATG- ATGAAACACTTTCCATCC	NdeI
	Rev	CGCGGATCC -ACCCACGTTGTAAGGTTG	BamHI
fu (961 )- 741(MC58)-His	Fwd	CGCGGATCC -GGAGGGGGTGGTGTCTG	BamHI
	Rev	CCCGCTCGAG-TTGCTTGGCGGCAAGGC	XhoI
fu (961 )-983-His	Fwd	CGCGGATCC - GGCGGAGGCGGCACTT	BamHI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
fu (961)- Orf46.1- His	Fwd	CGCGGATCCGGTGGTGGTGGT- TCAGATTGGCAAACGATTC	BamHI
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
fu (961 c-L)- 741(MC58)	Fwd	CGCGGATCC -GGAGGGGGTGGTGTCTG	BamHI
	Rev	CCCGCTCGAG-TTATTGCTTGGCGGCAAG	XhoI
fu (961c-L )-983	Fwd	CGCGGATCC - GGCGGAGGCGGCACTT	BamHI
	Rev	CCCGCTCGAG-TCAGAACCGGTAGCCTAC	XhoI
fu (961c-L)- Orf46.1	Fwd	CGCGGATCCGGTGGTGGTGGT- TCAGATTGGCAAACGATTC	BamHI
	Rev	CCCGCTCGAG-TTACGTATCATATTTACGTGC	XhoI
961-(His/GST)	Fwd	CGCGGATCCCATATG-GCCACAAGCGACGACG	BamHI-NdeI

(MC58)	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI
961 Δ1-His	Fwd	CGCGGATCCC <u>CATATG</u> -GCCACAAACGACGAC	NdeI
	Rev	CCCGCTCGAG-TGCTTTGGCGGCAAAGTT	XhoI
961a-(His/GST)	Fwd	CGCGGATCCC <u>CATATG</u> -GCCACAAACGACGAC	BamHI-NdeI
	Rev	CCCGCTCGAG-TTTAGCAATATTATCTTTGTTTCGTAGC	XhoI
961b-(His/GST)	Fwd	CGCGGATCCC <u>CATATG</u> -AAAGCAAACCGTGCCGA	BamHI-NdeI
	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI
961-His/GST <sup>GATE</sup>	Fwd	ggggacaagttgtacaaaaagcaggctGCAGCCACAAACGACGACG ATGTTAAAAAAGC	attB1
	Rev	ggggaccactttgtacaagaagctgggtTTACCACTCGTAATTGACGC CGACATGGTAGG	attB2
982	Fwd	GCGGCCATATG-GCAGCAAAAGACGTACAGTT	NdeI
	Rev	GCGGCCTCGAG-TTACATCATGCCGCCCATAACCA	XhoI
983-His (2996)	Fwd	CGCGGATCCGCTAGC-TTAGGCGGCGGCGGAG	NheI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
ΔG983-His (2996)	Fwd	CCCCTAGCTAGC-ACCTTCTGCGCCCGACTT	NheI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
983-His	Fwd	CGCGGATCCGCTAGC-TTAGGCGGCGGCGGAG	NheI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
ΔG983-His	Fwd	CGCGGATCCGCTAGC-ACCTTCTGCGCCCGACTT	NheI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
983L	Fwd	CGCGGATCCGCTAGC- CGAACGACCCCAACCTTCCCTACAAAACTTTCAA	NheI
	Rev	CCCGCTCGAG-TCAGAACCGACGTGCCAAGCCGTTT	XhoI
987-His (MC58)	Fwd	GCCGCCATATGCCCCCACTGGAAGAACGGACG	NdeI
	Rev	GCCGCCTCGAGTAATAAACCTTCTATGGGCAGCAG	XhoI
989-(His/GST) (MC58)	Fwd	CGCGGATCCC <u>CATATG</u> -TCCGTCCACGCATCCG	BamHI-NdeI
	Rev	CCCGCTCGAG-TTTGAATTTGTAGGTGTATTG	XhoI
989L (MC58)	Fwd	CGCGGATCCC <u>CATATG</u> -ACCCCTTCCGCACT	NdeI
	Rev	CCCGCTCGAG-TTATTTGAATTTGTAGGTGTAT	XhoI
CrgA-His (MC58)	Fwd	CGCGGATCCC <u>CATATG</u> -AAAACCAATTCAGAAGAA	NdeI
	Rev	CCCGCTCGAG-TCCACAGAGATTGTTTCC	XhoI
PilC1-ES (MC58)	Fwd	GATGCCCGAAGGGCGGG	
	Rev	GCCCAAGCTT-TCAGAAGAAGACTTCACGC	
PilC1-His (MC58)	Fwd	CGCGGATCCC <u>CATATG</u> -CAAACCCATAAATACGCTATT	NdeI
	Rev	GCCCAAGCTT-GAAGAAGACTTCACGCCAG	HindIII
Δ1PilC1-His (MC58)	Fwd	CGCGGATCCC <u>CATATG</u> -GTCTTTTTCGACAATACCGA	NdeI
	Rev	GCCCAAGCTT-	HindIII
PilC1L (MC58)	Fwd	CGCGGATCCC <u>CATATG</u> -AATAAACTTTAAAAAGGCGG	NdeI
	Rev	GCCCAAGCTT-TCAGAAGAAGACTTCACGC	HindIII
ΔGTbp2-His (MC58)	Fwd	CGCGAATCCC <u>CATATG</u> -TTCGATCTTGATTCTGTCTGA	NdeI
	Rev	CCCGCTCGAG-TCGCACAGGCTGTTGGCG	XhoI
Tbp2-His (MC58)	Fwd	CGCGAATCCC <u>CATATG</u> -TTGGGCGGAGGCGGCAG	NdeI
	Rev	CCCGCTCGAG-TCGCACAGGCTGTTGGCG	XhoI
Tbp2-His(MC58)	Fwd	CGCGAATCCC <u>CATATG</u> -TTGGGCGGAGGCGGCAG	NdeI
	Rev	CCCGCTCGAG-TCGCACAGGCTGTTGGCG	XhoI

NMB0109- (His/GST) (MC58)	Fwd	CGCGGATCCCATATG-GCAAATTTGGAGGTGCGC	BamHI-NdeI
	Rev	CCCCTCGAG-TTCGGAGCGGTTGAAGC	XhoI
NMB0109L (MC58)	Fwd	CGCGGATCCCATATG-CAACGTCGTATTATAACCC	NdeI
	Rev	CCCCTCGAG-TTATTCGGAGCGGTTGAAG	XhoI
NMB0207- (His/GST) (MC58)	Fwd	CGCGGATCCCATATG-GGCATCAAAGTCGCCATCAACGGCTAC	BamHI-NdeI
	Rev	CCCCTCGAG-TTTGAGCGGGCGCACTTCAAGTCCG	XhoI
NMB0462- (His/GST) (MC58)	Fwd	CGCGGATCCCATATG-GGCGGCAGCGAAAAAAC	BamHI-NdeI
	Rev	CCCCTCGAG-GTTGGTGCCGACTTTGAT	XhoI
NMB0623- (His/GST) (MC58)	Fwd	CGCGGATCCCATATG-GGCGGCGGAAGCGATA	BamHI-NdeI
	Rev	CCCCTCGAG-TTTGCCCGCTTTGAGCC	XhoI
NMB0625 (His- GST)(MC58)	Fwd	CGCGGATCCCATATGGGCAAATCCGAAAATACG	BamHI-NdeI
	Rev	CCCCTCGAGCATCCCGTACTGTTTCG	XhoI
NMB0634 (His/GST)(MC58)	Fwd	ggggacaagttgtacaaaaagcaggctCCGACATTACCGTGTACAAC GGCCAACAAAGAA	attB1
	Rev	ggggaccactttgtacaagaagctgggtCTTATTTTCATACCGGCTTGCT CAAGCAGCCGG	attB2
NMB0776- His/GST (MC58) GATE	Fwd	ggggacaagttgtacaaaaagcaggctGATACGGTGTTCCTGTAA AACGGACAACAA	attB1
	Rev	ggggaccactttgtacaagaagctgggtCTAGGAAAAATCGTCATCGT TGAAATTCGCC	attB2
NMB1115- His/GST (MC58) GATE	Fwd	ggggacaagttgtacaaaaagcaggctATGCACCCCATCGAAACC	attB1
	Rev	ggggaccactttgtacaagaagctgggtCTAGTCTGCAGTGCCTC	attB2
NMB1343- (His/GST) (MC58)	Fwd	CGCGGATCCCATATG-GGAAATTTCTTATATAGAGGCATTAG	BamHI-NdeI
	Rev	CCCCTCGAG-GTTAATTTCTATCAACTCTTTAGCAATAAT	XhoI
NMB1369 (His- GST) (MC58)	Fwd	CGCGGATCCCATATGGCCTGCCAAGACGACA	BamHI-NdeI
	Rev	CCCCTCGAGCCGCCTCTGCCGAAA	XhoI
NMB1551 (His- GST)(MC58)	Fwd	CGCGGATCCCATATGGCAGAGATCTGTTTGATAA	BamHI-NdeI
	Rev	CCCCTCGAGCGGTTTTCCGCCCAATG	XhoI
NMB1899 (His- GST) (MC58)	Fwd	CGCGGATCCCATATGCAGCCGGATACGGTC	BamHI-NdeI
	Rev	CCCCTCGAGAATCACTTCCAACACAAAAT	XhoI
NMB2050- (His/GST) (MC58)	Fwd	CGCGGATCCCATATG-TGGTTGCTGATGAAGGGC	BamHI-NdeI
	Rev	CCCCTCGAG-GACTGCTTCATCTTCTGC	XhoI
NMB2050L (MC58)	Fwd	CGCGGATCCCATATG-GAACTGATGACTGTTTTGC	NdeI
	Rev	CCCCTCGAG-TCAGACTGCTTCATCTTCT	XhoI
NMB2159- (His/GST) (MC58)	Fwd	CGCGGATCCCATATG-AGCATTAAGTAGCGATTAACGGTTTCGGC	BamHI-NdeI
	Rev	CCCCTCGAG-GATTTTGCTGCGAAGTATTCCAAAGTGCG	XhoI
fu-AG287...-His	Fwd	CGCGGATCCGCTAGC-CCCGATGTAAATCGGC	NheI

	Rev	CGGGGATCC-ATCCTGCTCTTTTTTGCCGG	BamHI
fu-(ΔG287)-919-His	Fwd	CGCGGATCCGGTGGTGGTGGT-CAAAGCAAGAGCATCCAAACC	BamHI
	Rev	CCCAAGCTT-TTCGGGCGGTATTCGGGCTTC	HindIII
fu-(ΔG287)-953-His	Fwd	CGCGGATCCGGTGGTGGTGGT-GCCACCTACAAAGTGGAC	BamHI
	Rev	GCCCAAGCTT-TGTTTGGCTGCCTCGAT	HindIII
fu-(ΔG287)-961-His	Fwd	CGCGGATCCGGTGGTGGTGGT-ACAAGCGACGACG	BamHI
	Rev	GCCCAAGCTT-CCACTCGTAATTGACGCC	HindIII
fu-(ΔG287)-Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGTGGT-TCAGATTGGCAAACGATTC	BamHI
	Rev	CCCAAGCTT-CGTATCATATTTACGTGC	HindIII
fu-(ΔG287-919)-Orf46.1-His	Fwd	CCCAAGCTTGGTGGTGGTGGTGGT-TCAGATTGGCAAACGATTC	HindIII
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
fu-(ΔG287-Orf46.1)-919-His	Fwd	CCCAAGCTTGGTGGTGGTGGTGGT-CAAAGCAAGAGCATCCAAACC	HindIII
	Rev	CCCGCTCGAG-CGGGCGGTATTCGGGCTT	XhoI
fu ΔG287(394.98)-...	Fwd	CGCGGATCCGCTAGC-CCCGATGTAAATCGGC	NheI
	Rev	CGGGGATCC-ATCCTGCTCTTTTTTGCCGG	BamHI
fu Orf1-(Orf46.1)-His	Fwd	CGCGGATCCGCTAGC-GGACACACTTATTTCCGGCATC	NheI
	Rev	CGCGGATCC-CCAGCGGTAGCCTAATTTGAT	
fu (Orf1)-Orf46.1-His	Fwd	CGCGGATCCGGTGGTGGTGGT-TCAGATTGGCAAACGATTC	BamHI
	Rev	CCCAAGCTT-CGTATCATATTTACGTGC	HindIII
fu (919)-Orf46.1-His	Fwd1	GCGGCGTTCGACGGTGGCGGAGGCACTGGATCCTCAG	SalI
	Fwd2	GGAGGCACTGGATCCTCAGATTGGCAAACGATTC	
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
Fu orf46-....	Fwd	GGAATTCATATGTCAGATTGGCAAACGATTC	NdeI
	Rev	CGCGGATCCCGTATCATATTTACGTGC	BamHI
Fu (orf46)-287-His	Fwd	CGGGGATCCGGGGGCGGCGGTGGCG	BamHI
	Rev	CCCAAGCTTATCCTGCTCTTTTTTGCCGGC	HindIII
Fu (orf46)-919-His	Fwd	CGCGGATCCGGTGGTGGTGGTCAAAGCAAGAGCATCCAACC	BamHI
	Rev	CCCAAGCTTCGGGCGGTATTCGGGCTTC	HindIII
Fu (orf46-919)-287-His	Fwd	CCCAAGCTTGGGGGCGGCGGTGGCG	HindIII
	Rev	CCCGCTCGAGATCCTGCTCTTTTTTGCCGGC	XhoI
Fu (orf46-287)-919-His	Fwd	CCCAAGCTTGGTGGTGGTGGTGGTCAAAGCAAGAGCATCCAAACC	HindIII
	Rev	CCCGCTCGAGCGGGCGGTATTCGGGCTT	XhoI
(ΔG741)-961c-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-ACCCAGCTTGTAAGGTTG	XhoI
(ΔG741)-961-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCCTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI

(ΔG741)-983-His	Fwd	GCGGCCTCGAG-GGATCCGGCGGAGGCGGCACTTCTGCG	XhoI
	Rev	CCCGCTCGAG-GAACCGGTAGCCTACG	XhoI
(ΔG741)-orf46.1-His	Fwd1	GGAGGCACTGGATCCTCAGATTTGGCAAACGATTTC	SalI
	Fwd2	GCGGCGTTCGACGGTGGCGGAGGCACTGGATCCTCAGA	
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI
(ΔG983)-741(MC58)-His	Fwd	GCGGCCTCGAG-GGATCCGGAGGGGGTGGTGTGCGCC	XhoI
	Rev	CCCGCTCGAG-TTGCTTGGCGGCAAG	XhoI
(ΔG983)-961c-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCGTTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-ACCCAGCTTGTAAGGTTG	XhoI
(ΔG983)-961-His	Fwd1	GGAGGCACTGGATCCGCAGCCACAAACGACGACGA	XhoI
	Fwd2	GCGGCGTTCGAG-GGTGGCGGAGGCACTGGATCCGCAG	
	Rev	CCCGCTCGAG-CCACTCGTAATTGACGCC	XhoI
(ΔG983)-Orf46.1-His	Fwd1	GGAGGCACTGGATCCTCAGATTTGGCAAACGATTTC	SalI
	Fwd2	GCGGCGTTCGACGGTGGCGGAGGCACTGGATCCTCAGA	
	Rev	CCCGCTCGAG-CGTATCATATTTACGTGC	XhoI

\* This primer was used as a Reverse primer for all the C terminal fusions of 287 to the His-tag.

§ Forward primers used in combination with the 287-His Reverse primer.

NB – All PCR reactions use strain 2996 unless otherwise specified (e.g. strain MC58)

In all constructs starting with an ATG not followed by a unique *NheI* site, the ATG codon is part of the *NdeI* site used for cloning. The constructs made using *NheI* as a cloning site at the 5' end (e.g. all those containing 287 at the N-terminus) have two additional codons (GCT AGC) fused to the coding sequence of the antigen.

### *Preparation of chromosomal DNA templates*

*N.meningitidis* strains 2996, MC58, 394.98, 1000 and BZ232 (and others) were grown to exponential phase in 100ml of GC medium, harvested by centrifugation, and resuspended in 5ml buffer (20% w/v sucrose, 50mM Tris-HCl, 50mM EDTA, pH8). After 10 minutes incubation on ice, the bacteria were lysed by adding 10ml of lysis solution (50mM NaCl, 1% Na-Sarkosyl, 50μg/ml Proteinase K), and the suspension incubated at 37°C for 2 hours. Two phenol extractions (equilibrated to pH 8) and one CHCl<sub>3</sub>/isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes of ethanol, and collected by centrifugation. The pellet was washed once with 70%(v/v) ethanol and redissolved in 4.0ml TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). The DNA concentration was measured by reading OD<sub>260</sub>.

### ***PCR Amplification***

The standard PCR protocol was as follows: 200ng of genomic DNA from 2996, MC581000, or BZ232 strains or 10ng of plasmid DNA preparation of recombinant clones were used as template in the presence of 40µM of each oligonucleotide primer, 400-800 µM dNTPs solution, 1x PCR buffer (including 1.5mM MgCl<sub>2</sub>), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaq, Boehringer Mannheim Expand™ Long Template).

After a preliminary 3 minute incubation of the whole mix at 95°C, each sample underwent a two-step amplification: the first 5 cycles were performed using the hybridisation temperature that excluded the restriction enzyme tail of the primer (T<sub>m1</sub>). This was followed by 30 cycles according to the hybridisation temperature calculated for the whole length oligos (T<sub>m2</sub>). Elongation times, performed at 68°C or 72°C, varied according to the length of the Orf to be amplified. In the case of Orf1 the elongation time, starting from 3 minutes, was increased by 15 seconds each cycle. The cycles were completed with a 10 minute extension step at 72°C.

The amplified DNA was either loaded directly on a 1% agarose gel. The DNA fragment corresponding to the band of correct size was purified from the gel using the Qiagen Gel Extraction Kit, following the manufacturer's protocol.

### ***Digestion of PCR fragments and of the cloning vectors***

The purified DNA corresponding to the amplified fragment was digested with the appropriate restriction enzymes for cloning into pET-21b+, pET22b+ or pET-24b+. Digested fragments were purified using the QIAquick PCR purification kit (following the manufacturer's instructions) and eluted with either H<sub>2</sub>O or 10mM Tris, pH 8.5. Plasmid vectors were digested with the appropriate restriction enzymes, loaded onto a 1.0% agarose gel and the band corresponding to the digested vector purified using the Qiagen QIAquick Gel Extraction Kit.

### ***Cloning***

The fragments corresponding to each gene, previously digested and purified, were ligated into pET21b+, pET22b+ or pET-24b+. A molar ratio of 3:1 fragment/vector was used with T4 DNA ligase in the ligation buffer supplied by the manufacturer.

Recombinant plasmid was transformed into competent *E.coli* DH5 or HB101 by incubating the ligase reaction solution and bacteria for 40 minutes on ice, then at 37°C for 3 minutes.

This was followed by the addition of 800µl LB broth and incubation at 37°C for 20 minutes. The cells were centrifuged at maximum speed in an Eppendorf microfuge, resuspended in approximately 200µl of the supernatant and plated onto LB ampicillin (100mg/ml) agar.

Screening for recombinant clones was performed by growing randomly selected colonies overnight at 37°C in 4.0ml of LB broth + 100µg/ml ampicillin. Cells were pelleted and plasmid DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions. Approximately 1µg of each individual miniprep was digested with the appropriate restriction enzymes and the digest loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1kb DNA Ladder, GIBCO). Positive clones were selected on the basis of the size of insert.

### ***Expression***

After cloning each gene into the expression vector, recombinant plasmids were transformed into *E.coli* strains suitable for expression of the recombinant protein. 1µl of each construct was used to transform *E.coli* BL21-DE3 as described above. Single recombinant colonies were inoculated into 2ml LB+Amp (100µg/ml), incubated at 37°C overnight, then diluted 1:30 in 20ml of LB+Amp (100µg/ml) in 100ml flasks, to give an OD<sub>600</sub> between 0.1 and 0.2. The flasks were incubated at 30°C or at 37°C in a gyratory water bath shaker until OD<sub>600</sub> indicated exponential growth suitable for induction of expression (0.4-0.8 OD). Protein expression was induced by addition of 1.0mM IPTG. After 3 hours incubation at 30°C or 37°C the OD<sub>600</sub> was measured and expression examined. 1.0ml of each sample was centrifuged in a microfuge, the pellet resuspended in PBS and analysed by SDS-PAGE and Coomassie Blue staining.

### ***Gateway cloning and expression***

Sequences labelled GATE were cloned and expressed using the GATEWAY Cloning Technology (GIBCO-BRL). Recombinational cloning (RC) is based on the recombination reactions that mediate the integration and excision of phage into and from the *E.coli* genome, respectively. The integration involves recombination of the *attP* site of the phage DNA within the *attB* site located in the bacterial genome (BP reaction) and generates an integrated phage genome flanked by *attL* and *attR* sites. The excision recombines *attL* and *attR* sites back to *attP* and *attB* sites (LR reaction). The integration reaction requires two enzymes [the phage protein Integrase (Int) and the bacterial protein integration host factor (IHF)] (BP clonase). The

- excision reaction requires Int, IHF, and an additional phage enzyme, Excisionase (Xis) (LR clonase). Artificial derivatives of the 25-bp bacterial *attB* recombination site, referred to as B1 and B2, were added to the 5' end of the primers used in PCR reactions to amplify Neisserial ORFs. The resulting products were BP cloned into a "Donor vector" containing complementary derivatives of the phage *attP* recombination site (P1 and P2) using BP clonase. The resulting "Entry clones" contain ORFs flanked by derivatives of the *attL* site (L1 and L2) and were subcloned into expression "destination vectors" which contain derivatives of the *attL*-compatible *attR* sites (R1 and R2) using LR clonase. This resulted in "expression clones" in which ORFs are flanked by B1 and B2 and fused in frame to the GST or His N terminal tags.
- 10 The *E. coli* strain used for GATEWAY expression is BL21-SI. Cells of this strain are induced for expression of the T7 RNA polymerase by growth in medium containing salt (0.3 M NaCl).

Note that this system gives N-terminus His tags.

#### ***Preparation of membrane proteins.***

- Fractions composed principally of either inner, outer or total membrane were isolated in order to obtain recombinant proteins expressed with membrane-localisation leader sequences. The method for preparation of membrane fractions, enriched for recombinant proteins, was adapted from Filip *et. al.* [*J.Bact.* (1973) 115:717-722] and Davies *et. al.* [*J.Immunol.Meth.* (1990) 143:215-225]. Single colonies harbouring the plasmid of interest were grown overnight at 37°C in 20 ml of LB/Amp (100 µg/ml) liquid culture. Bacteria were diluted 1:30 in 1.0 L of fresh medium and grown at either 30°C or 37°C until the OD<sub>550</sub> reached 0.6-0.8. Expression of recombinant protein was induced with IPTG at a final concentration of 1.0 mM. After incubation for 3 hours, bacteria were harvested by centrifugation at 8000g for 15 minutes at 4°C and resuspended in 20 ml of 20 mM Tris-HCl (pH 7.5) and complete protease inhibitors (Boehringer-Mannheim). All subsequent procedures were performed at 4°C or on ice.

- Cells were disrupted by sonication using a Branson Sonifier 450 and centrifuged at 5000g for 20 min to sediment unbroken cells and inclusion bodies. The supernatant, containing membranes and cellular debris, was centrifuged at 50000g (Beckman Ti50, 29000rpm) for 75 min, washed with 20 mM Bis-tris propane (pH 6.5), 1.0 M NaCl, 10% (v/v) glycerol and sedimented again at 50000g for 75 minutes. The pellet was resuspended in 20mM Tris-HCl (pH 7.5), 2.0% (v/v) Sarkosyl, complete protease inhibitor (1.0 mM EDTA, final

concentration) and incubated for 20 minutes to dissolve inner membrane. Cellular debris was pelleted by centrifugation at 5000g for 10 min and the supernatant centrifuged at 75000g for 75 minutes (Beckman Ti50, 33000rpm). Proteins 008L and 519L were found in the supernatant suggesting inner membrane localisation. For these proteins both inner and total  
5 membrane fractions (washed with NaCl as above) were used to immunise mice. Outer membrane vesicles obtained from the 75000g pellet were washed with 20 mM Tris-HCl (pH 7.5) and centrifuged at 75000g for 75 minutes or overnight. The OMV was finally resuspended in 500 µl of 20 mM Tris-HCl (pH 7.5), 10% v/v glycerol. Orf1L and Orf40L were both localised and enriched in the outer membrane fraction which was used to  
10 immunise mice. Protein concentration was estimated by standard Bradford Assay (Bio-Rad), while protein concentration of inner membrane fraction was determined with the DC protein assay (Bio-Rad). Various fractions from the isolation procedure were assayed by SDS-PAGE.

#### ***Purification of His-tagged proteins***

Various forms of 287 were cloned from strains 2996 and MC58. They were constructed with  
15 a C-terminus His-tagged fusion and included a mature form (aa 18-427), constructs with deletions ( $\Delta 1$ ,  $\Delta 2$ ,  $\Delta 3$  and  $\Delta 4$ ) and clones composed of either B or C domains. For each clone purified as a His-fusion, a single colony was streaked and grown overnight at 37°C on a LB/Amp (100 µg/ml) agar plate. An isolated colony from this plate was inoculated into 20ml of LB/Amp (100 µg/ml) liquid medium and grown overnight at 37°C with shaking.  
20 The overnight culture was diluted 1:30 into 1.0 L LB/Amp (100 µg/ml) liquid medium and allowed to grow at the optimal temperature (30 or 37°C) until the OD<sub>550</sub> reached 0.6-0.8. Expression of recombinant protein was induced by addition of IPTG (final concentration 1.0mM) and the culture incubated for a further 3 hours. Bacteria were harvested by centrifugation at 8000g for 15 min at 4°C. The bacterial pellet was resuspended in 7.5 ml of  
25 either (i) cold buffer A (300 mM NaCl, 50 mM phosphate buffer, 10 mM imidazole, pH 8.0) for soluble proteins or (ii) buffer B (10mM Tris-HCl, 100 mM phosphate buffer, pH 8.8 and, optionally, 8M urea) for insoluble proteins. Proteins purified in a soluble form included 287-His,  $\Delta 1$ ,  $\Delta 2$ ,  $\Delta 3$  and  $\Delta 4$ 287-His,  $\Delta 4$ 287MC58-His, 287c-His and 287cMC58-His. Protein 287bMC58-His was insoluble and purified accordingly. Cells were disrupted by  
30 sonication on ice four times for 30 sec at 40W using a Branson sonifier 450 and centrifuged at 13000xg for 30 min at 4°C. For insoluble proteins, pellets were resuspended in 2.0 ml buffer C (6 M guanidine hydrochloride, 100 mM phosphate buffer, 10 mM Tris- HCl, pH 7.5

and treated with 10 passes of a Dounce homogenizer. The homogenate was centrifuged at 13000g for 30 min and the supernatant retained. Supernatants for both soluble and insoluble preparations were mixed with 150µl Ni<sup>2+</sup>-resin (previously equilibrated with either buffer A or buffer B, as appropriate) and incubated at room temperature with gentle agitation for 30 min. The resin was Chelating Sepharose Fast Flow (Pharmacia), prepared according to the manufacturer's protocol. The batch-wise preparation was centrifuged at 700g for 5 min at 4°C and the supernatant discarded. The resin was washed twice (batch-wise) with 10ml buffer A or B for 10 min, resuspended in 1.0 ml buffer A or B and loaded onto a disposable column. The resin continued to be washed with either (i) buffer A at 4°C or (ii) buffer B at room temperature, until the OD<sub>280</sub> of the flow-through reached 0.02-0.01. The resin was further washed with either (i) cold buffer C (300mM NaCl, 50mM phosphate buffer, 20mM imidazole, pH 8.0) or (ii) buffer D (10mM Tris-HCl, 100mM phosphate buffer, pH 6.3 and, optionally, 8M urea) until OD<sub>280</sub> of the flow-through reached 0.02-0.01. The His-fusion protein was eluted by addition of 700µl of either (i) cold elution buffer A (300 mM NaCl, 50mM phosphate buffer, 250 mM imidazole, pH 8.0) or (ii) elution buffer B (10 mM Tris-HCl, 100 mM phosphate buffer, pH 4.5 and, optionally, 8M urea) and fractions collected until the OD<sub>280</sub> indicated all the recombinant protein was obtained. 20µl aliquots of each elution fraction were analysed by SDS-PAGE. Protein concentrations were estimated using the Bradford assay.

#### 20 *Renaturation of denatured His-fusion proteins.*

Denaturation was required to solubilize 287bMC8, so a renaturation step was employed prior to immunisation. Glycerol was added to the denatured fractions obtained above to give a final concentration of 10% v/v. The proteins were diluted to 200 µg/ml using dialysis buffer I (10% v/v glycerol, 0.5M arginine, 50 mM phosphate buffer, 5.0 mM reduced glutathione, 0.5 mM oxidised glutathione, 2.0M urea, pH 8.8) and dialysed against the same buffer for 12-14 hours at 4°C. Further dialysis was performed with buffer II (10% v/v glycerol, 0.5M arginine, 50mM phosphate buffer, 5.0mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was estimated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times OD_{280}) - (0.76 \times OD_{260})$$

### *Amino acid sequence analysis.*

Automated sequence analysis of the NH<sub>2</sub>-terminus of proteins was performed on a Beckman sequencer (LF 3000) equipped with an on-line phenylthiohydantoin-amino acid analyser (System Gold) according to the manufacturer's recommendations.

## 5 *Immunization*

Balb/C mice were immunized with antigens on days 0, 21 and 35 and sera analyzed at day 49.

### *Sera analysis – ELISA*

The acapsulated MenB M7 and the capsulated strains were plated on chocolate agar plates and incubated overnight at 37°C with 5% CO<sub>2</sub>. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.4-0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and bacteria were washed twice with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 1 hour at 37°C and then overnight at 4°C with stirring. 100µl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200µl of saturation buffer (2.7% polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200µl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN<sub>3</sub> in PBS) were added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 100µl of HRP-conjugated rabbit anti-mouse (Dako) serum diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100µl of substrate buffer for HRP (25ml of citrate buffer pH5, 10mg of O-phenildiamine and 10µl of H<sub>2</sub>O<sub>2</sub>) were added to each well and the plates were left at room temperature for 20 minutes. 100µl 12.5% H<sub>2</sub>SO<sub>4</sub> was added to each well and OD<sub>490</sub> was followed. The ELISA titers were calculated arbitrarily as the dilution of sera which gave an OD<sub>490</sub> value of 0.4 above the level of preimmune sera. The ELISA was considered positive when the dilution of sera with OD<sub>490</sub> of 0.4 was higher than 1:400.

## 30 *Sera analysis – FACS Scan bacteria binding assay*

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C with 5% CO<sub>2</sub>. Bacterial colonies were collected from the agar plates using

a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD<sub>620</sub>. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and  
5 the pellet was resuspended in blocking buffer (1% BSA in PBS, 0.4% NaN<sub>3</sub>) and centrifuged for 5 minutes at 4000rpm. Cells were resuspended in blocking buffer to reach OD<sub>620</sub> of 0.05. 100µl bacterial cells were added to each well of a Costar 96 well plate. 100µl of diluted (1:100, 1:200, 1:400) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000rpm, the supernatant  
10 aspirated and cells washed by addition of 200µl/well of blocking buffer in each well. 100µl of R-Phicoerytrin conjugated F(ab)<sub>2</sub> goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200µl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200µl/well of PBS, 0.25% formaldehyde. Samples were  
15 transferred to FACScan tubes and read. The condition for FACScan (Laser Power 15mW) setting were: FL2 on; FSC-H threshold:92; FSC PMT Voltage: E 01; SSC PMT: 474; Amp. Gains 6.1; FL-2 PMT: 586; compensation values: 0.

#### ***Sera analysis – bactericidal assay***

*N. meningitidis* strain 2996 was grown overnight at 37°C on chocolate agar plates (starting  
20 from a frozen stock) with 5% CO<sub>2</sub>. Colonies were collected and used to inoculate 7ml Mueller-Hinton broth, containing 0.25% glucose to reach an OD<sub>620</sub> of 0.05-0.08. The culture was incubated for approximately 1.5 hours at 37 degrees with shacking until the OD<sub>620</sub> reached the value of 0.23-0.24. Bacteria were diluted in 50mM Phosphate buffer pH 7.2 containing 10mM MgCl<sub>2</sub>, 10mM CaCl<sub>2</sub> and 0.5% (w/v) BSA (assay buffer) at the working  
25 dilution of 10<sup>5</sup> CFU/ml. The total volume of the final reaction mixture was 50 µl with 25 µl of serial two fold dilution of test serum, 12.5 µl of bacteria at the working dilution, 12.5 µl of baby rabbit complement (final concentration 25% ).

Controls included bacteria incubated with complement serum, immune sera incubated with bacteria and with complement inactivated by heating at 56°C for 30'. Immediately after the  
30 addition of the baby rabbit complement, 10µl of the controls were plated on Mueller-Hinton agar plates using the tilt method (time 0). The 96-wells plate was incubated for 1 hour at 37°C with rotation. 7µl of each sample were plated on Mueller-Hinton agar plates as spots, whereas 10µl of the controls were plated on Mueller-Hinton agar plates using the tilt method

(time 1). Agar plates were incubated for 18 hours at 37 degrees and the colonies corresponding to time 0 and time 1 were counted.

### *Sera analysis – western blots*

Purified proteins (500ng/lane), outer membrane vesicles (5µg) and total cell extracts (25µg) derived from MenB strain 2996 were loaded onto a 12% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, using transfer buffer (0.3% Tris base, 1.44% glycine, 20% (v/v) methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with mice sera diluted 1:200 in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labelled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

The OMVs were prepared as follows: *N. meningitidis* strain 2996 was grown overnight at 37 degrees with 5% CO<sub>2</sub> on 5 GC plates, harvested with a loop and resuspended in 10 ml of 20mM Tris-HCl pH 7.5, 2 mM EDTA. Heat inactivation was performed at 56°C for 45 minutes and the bacteria disrupted by sonication for 5 minutes on ice (50% duty cycle, 50% output, Branson sonifier 3 mm microtip). Unbroken cells were removed by centrifugation at 5000g for 10 minutes, the supernatant containing the total cell envelope fraction recovered and further centrifuged overnight at 50000g at the temperature of 4°C. The pellet containing the membranes was resuspended in 2% sarkosyl, 20mM Tris-HCl pH 7.5, 2 mM EDTA and incubated at room temperature for 20 minutes to solubilise the inner membranes. The suspension was centrifuged at 10000g for 10 minutes to remove aggregates, the supernatant was further centrifuged at 50000g for 3 hours. The pellet, containing the outer membranes was washed in PBS and resuspended in the same buffer. Protein concentration was measured by the D.C. Bio-Rad Protein assay (Modified Lowry method), using BSA as a standard.

Total cell extracts were prepared as follows: *N. meningitidis* strain 2996 was grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes.

### *961 domain studies*

Cellular fractions preparation Total lysate, periplasm, supernatant and OMV of *E.coli* clones expressing different domains of 961 were prepared using bacteria from over-night cultures or

after 3 hours induction with IPTG. Briefly, the periplasm were obtained suspending bacteria in saccarose 25% and Tris 50mM (pH 8) with polymyxine 100µg/ml. After 1hr at room temperature bacteria were centrifuged at 13000rpm for 15 min and the supernatant were collected. The culture supernatant were filtered with 0.2µm and precipitated with TCA 50%  
5 in ice for two hours. After centrifugation (30 min at 13000 rp) pellets were rinsed twice with ethanol 70% and suspended in PBS. The OMV preparation was performed as previously described. Each cellular fraction were analyzed in SDS-PAGE or in Western Blot using the polyclonal anti-serum raised against GST-961.

Adhesion assay Chang epithelial cells (Wong-Kilbourne derivative, clone 1-5c-4, human  
10 conjunctiva) were maintained in DMEM (Gibco) supplemented with 10% heat-inactivated FCS, 15mM L-glutamine and antibiotics.

For the adherence assay, sub-confluent culture of Chang epithelial cells were rinsed with PBS and treated with trypsin-EDTA (Gibco), to release them from the plastic support. The cells were then suspended in PBS, counted and dilute in PBS to  $5 \times 10^5$  cells/ml.

15 Bacteria from over-night cultures or after induction with IPTG, were pelleted and washed twice with PBS by centrifuging at 13000 for 5 min. Approximately  $2-3 \times 10^8$  (cfu) were incubated with 0.5 mg/ml FITC (Sigma) in 1ml buffer containing 50mM  $\text{NaHCO}_3$  and 100mM NaCl pH 8, for 30 min at room temperature in the dark. FITC-labeled bacteria were wash 2-3 times and suspended in PBS at  $1-1.5 \times 10^9$ /ml. 200µl of this suspension ( $2-3 \times 10^8$ )  
20 were incubated with 200µl ( $1 \times 10^5$ ) epithelial cells for 30min a 37°C. Cells were than centrifuged at 2000rpm for 5 min to remove non-adherent bacteria, suspended in 200µl of PBS, transferred to FACScan tubes and read

**CLAIMS**

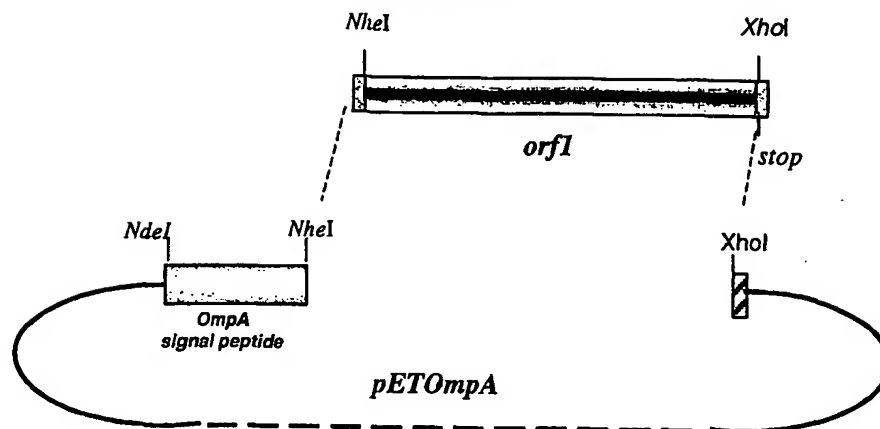
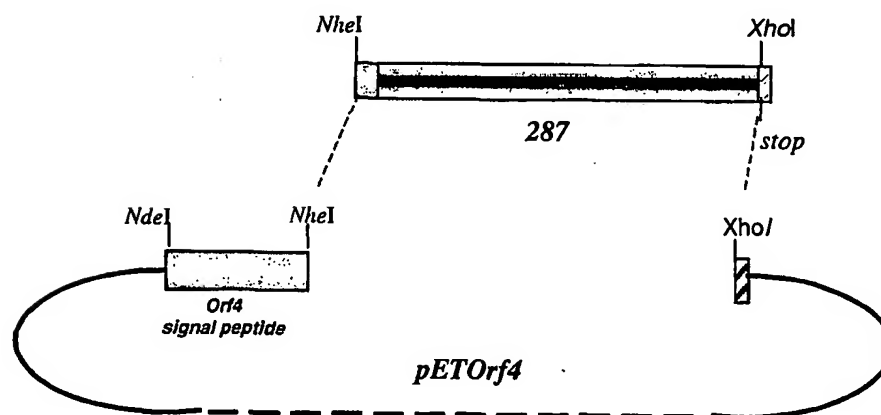
1. A method for the heterologous expression of a protein of the invention, in which (a) at least one domain in the protein is deleted and, optionally, (b) no fusion partner is used.
2. The method of claim 1, in which the protein of the invention is ORF46.
- 5 3. The method of claim 2, in which ORF46 is divided into a first domain (amino acids 1-433) and a second domain (amino acids 433-608).
4. The method of claim 2, in which the protein of the invention is 564.
5. The method of claim 4, in which protein 564 is divided into domains as shown in Figure 8.
- 10 6. The method of claim 1 in which the protein of the invention is 961.
7. The method of claim 6, in which protein 961 is divided into domains as shown in Figure 12.
8. The method of claim 1, in which the protein of the invention is 502 and the domain is amino acids 28 to 167 (numbered according to the MC58 sequence).
- 15 9. The method of claim 1, in which the protein of the invention is 287.
10. A method for the heterologous expression of a protein of the invention, in which (a) a portion of the N-terminal domain of the protein is deleted.
11. The method of claim 9 or claim 10, in which protein 287 is divided into domains A B & C shown in Figure 5.
- 20 12. The method of claim 11, in which (i) domain A, (ii) domains A and B, or (iii) domains A and C are deleted.
13. The method of claim 11, wherein (i) amino acids 1-17, (ii) amino acids 1-25, (iii) amino acids 1-69, or (iv) amino acids 1-106, of domain A are deleted.
- 25 14. A method for the heterologous expression of a protein of the invention, in which (a) no fusion partner is used, and (b) the protein's native leader peptide (if present) is used.

15. The method of claim 14, in which the protein of the invention is selected from the group consisting of: 111, 149, 206, 225-1, 235, 247-1, 274, 283, 286, 292, 401, 406, 502-1, 503, 519-1, 525-1, 552, 556, 557, 570, 576-1, 580, 583, 664, 759, 907, 913, 920-1, 936-1, 953, 961, 983, 989, Orf4, Orf7-1, Orf9-1, Orf23, Orf25, Orf37, Orf38, Orf40, Orf40.1, Orf40.2, Orf72-1, Orf76-1, Orf85-2, Orf91, Orf97-1, Orf119, Orf143.1, NMB0109, NMB2050, 008, 105, 117-1, 121-1, 122-1, 128-1, 148, 216, 243, 308, 593, 652, 726, 926, 982, Orf83-1 and Orf143-1.
16. A method for the heterologous expression of a protein of the invention, in which (a) the protein's leader peptide is replaced by the leader peptide from a different protein and, optionally, (b) no fusion partner is used.
17. The method of claim 16, in which the different protein is 961, ORF4, *E.coli* OmpA, or *E.carotovora* PelB, or in which the leader peptide is MKKYLFSAA.
18. The method of claim 17, in which the different protein is *E.coli* OmpA and the protein of the invention is ORF1.
19. The method of claim 17, in which the protein of the invention is 911 and the different protein is *E.carotovora* PelB or *E.coli* OmpA.
20. The method of claim 17, in which the different protein is ORF4 and the protein of the invention is 287.
21. A method for the heterologous expression of a protein of the invention, in which (a) the protein's leader peptide is deleted and, optionally, (b) no fusion partner is used.
22. The method of claim 21, in which the protein of the invention is 919.
23. A method for the heterologous expression of a protein of the invention, in which expression of a protein of the invention is carried out at a temperature at which a toxic activity of the protein is not manifested.
24. The method of claim 23, in which protein 919 is expressed at 30°C.
25. A method for the heterologous expression of a protein of the invention, in which protein is mutated to reduce or eliminate toxic activity.

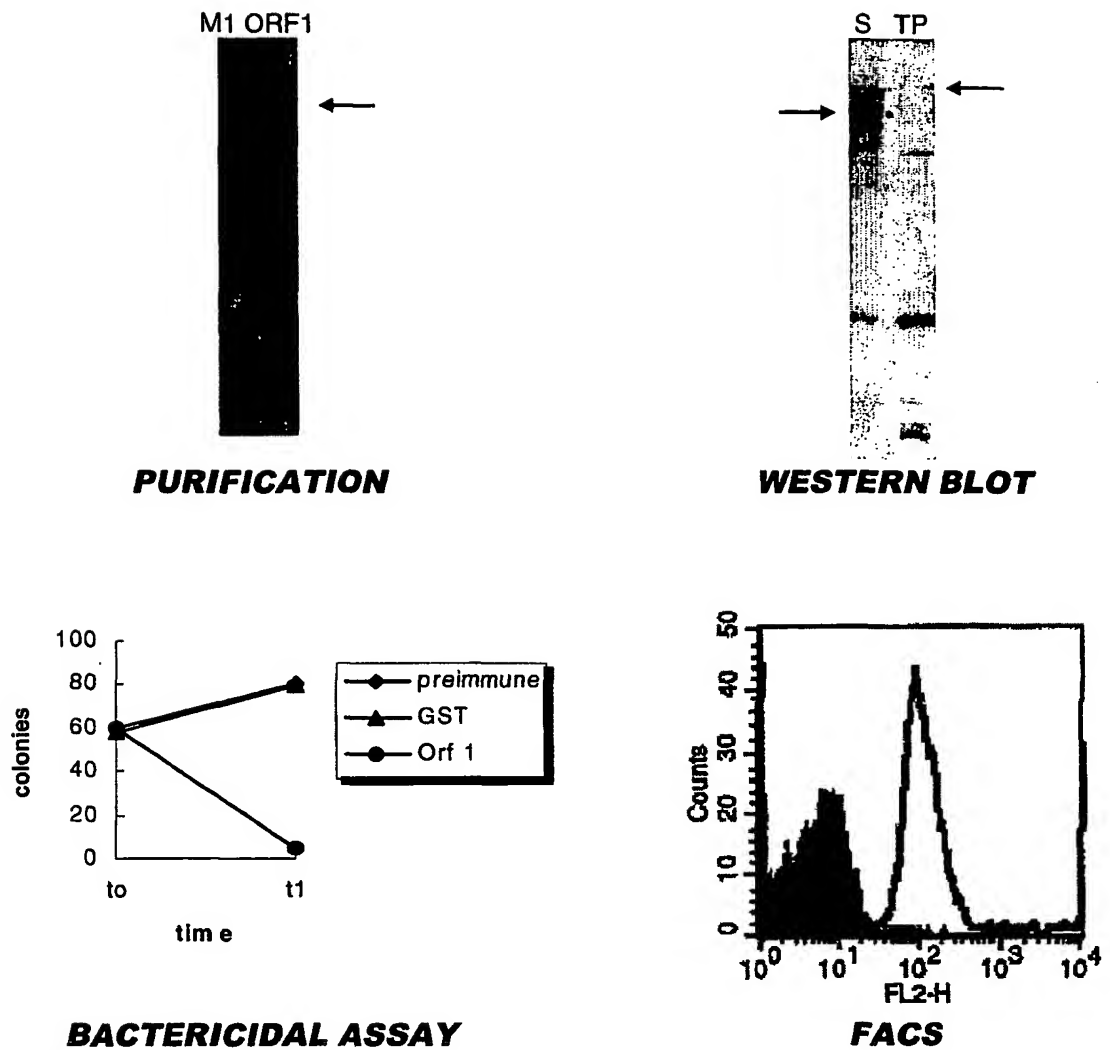
26. The method of claim 25, in which the protein of the invention is 907, 919 or 922.
27. The method of claim 26, in which 907 is mutated at Glu-117 (*e.g.* Glu→Gly).
28. The method of claim 26, in which 919 is mutated at Glu-255 (*e.g.* Glu→Gly) and/or Glu-323 (*e.g.* Glu→Gly).
- 5 29. The method of claim 26, in which 922 is mutated at Glu-164 (*e.g.* Glu→Gly), Ser-213 (*e.g.* Ser→Gly) and/or Asn-348 (*e.g.* Asn→Gly).
30. A method for the heterologous expression of a protein of the invention, in which vector pSM214 is used or vector pET-24b is used.
- 10 31. The method of claim 30, in which the protein of the invention is 953 and the vector is pSM214.
32. A method for the heterologous expression of a protein of the invention, in which a protein is expressed or purified such that it adopts a particular multimeric form.
33. The method of claim 32, in which protein 953 is expressed and/or purified in monomeric form.
- 15 34. The method of claim 32, in which protein 961 is expressed and/or purified in tetrameric form.
35. The method of claim 32, in which protein 287 is expressed and/or purified in dimeric form.
- 20 36. The method of claim 32, in which protein 919 is expressed and/or purified in monomeric form.
37. A method for the heterologous expression of a protein of the invention, in which the protein is expressed as a lipidated protein.
38. The method of claim 37, in which the protein of the invention is 919, 287, ORF4, 406, 576, or ORF25.
- 25 39. A method for the heterologous expression of a protein of the invention, in which (a) the protein's C-terminus region is mutated and, optionally, (b) no fusion partner is used.

40. The method of claim 39, wherein the mutation is a substitution, an insertion, or a deletion
41. The method of claim 40, wherein the protein of the invention is 730, ORF29 or ORF46.
42. A method for the heterologous expression of a protein of the invention, in which the protein's leader peptide is mutated.
- 5 43. The method of claim 42, in which the protein of the invention is 919.
44. A method for the heterologous expression of a protein, in which a poly-glycine stretch within the protein is mutated.
45. The method of claim 44, wherein the protein is a protein of the invention.
46. The method of claim 45, wherein the protein of the invention is 287, 741, 983 or Tbp2.
- 10 47. The method of claim 46, wherein (Gly)<sub>6</sub> is deleted from 287 or 983.
48. The method of claim 46, wherein (Gly)<sub>4</sub> is deleted from Tbp2 or 741
49. The method of claim 47 or claim 48, wherein the leader peptide is also deleted.
50. The method of any preceding claim, in which the heterologous expression is in an *E.coli* host.
- 15 51. A protein expressed by the method of any preceding claim.
52. A heterologous protein comprising the N-terminal amino acid sequence MKKYLFSA.

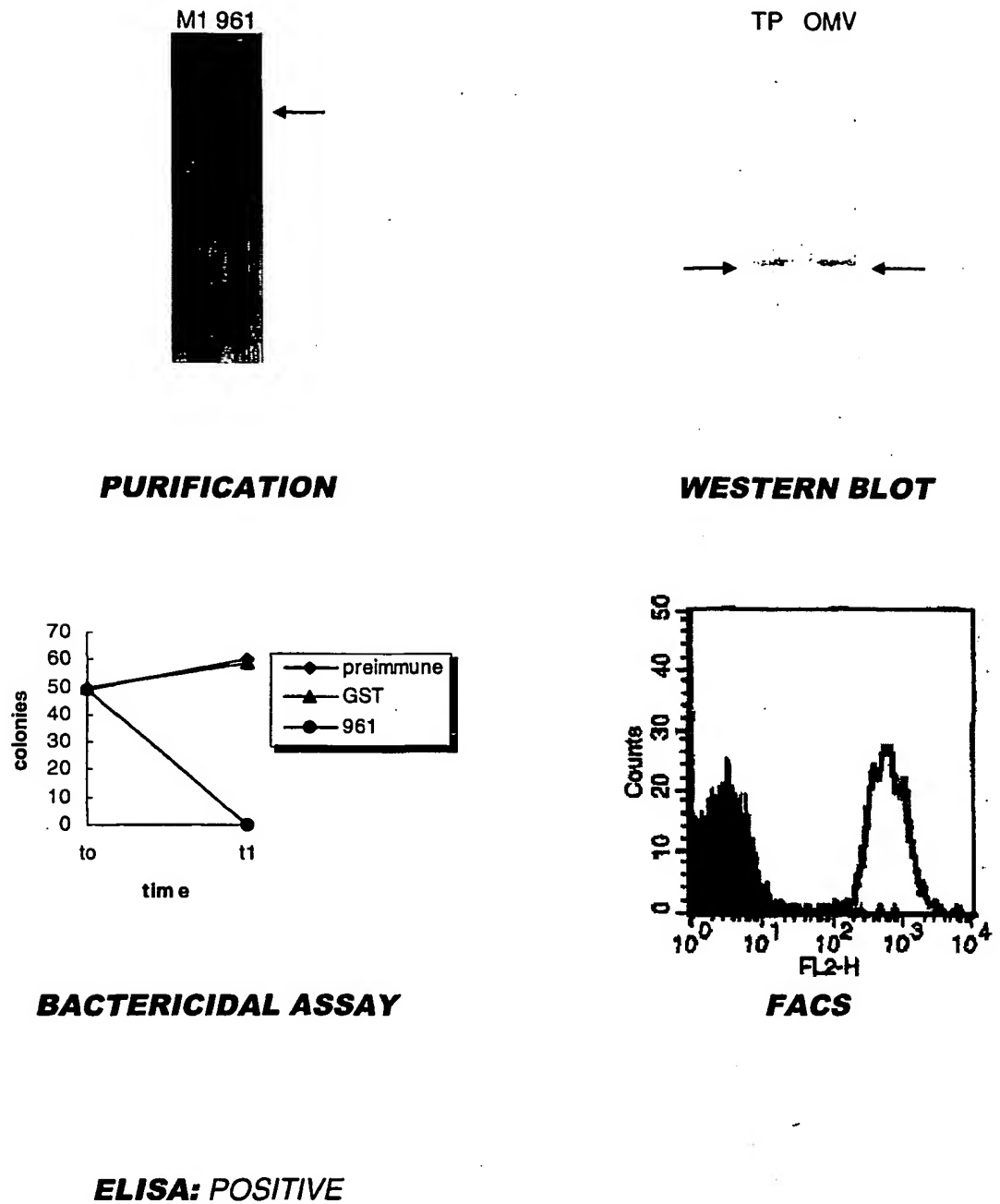
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**FIGURE 1****FIGURE 2**

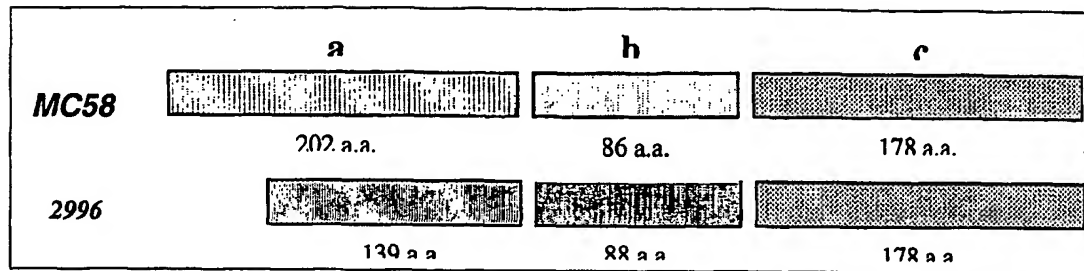
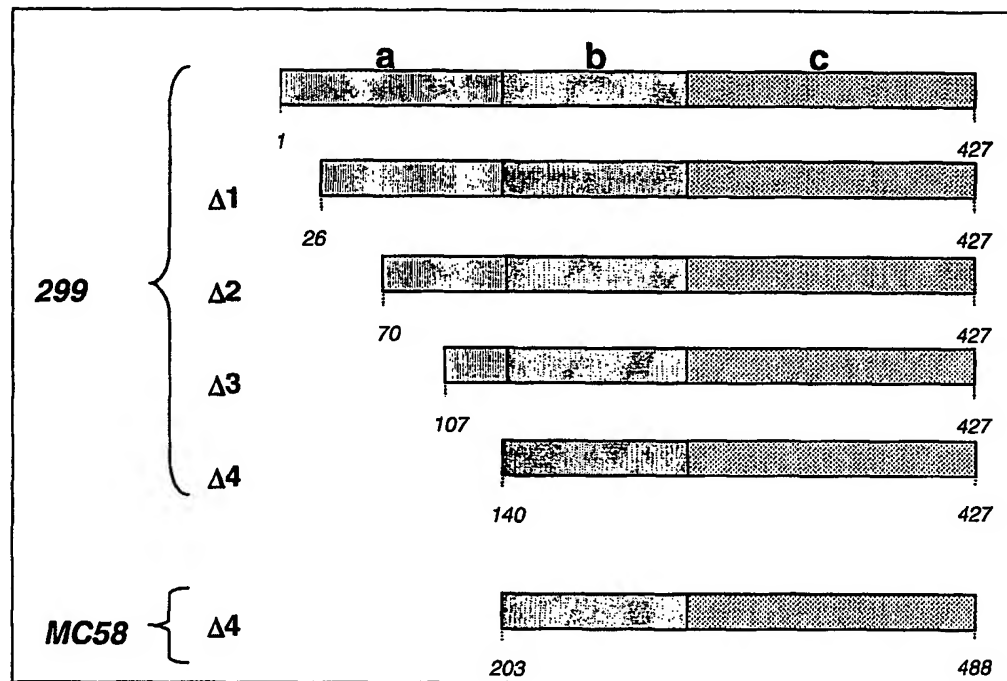
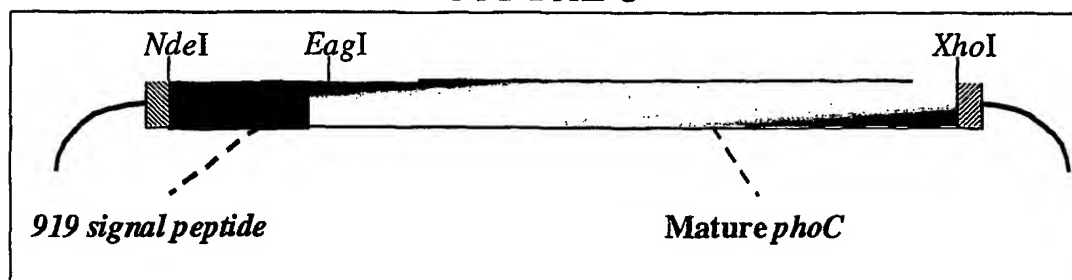
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**FIGURE 3****ELISA: POSITIVE**

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**FIGURE 4**

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**FIGURE 5****FIGURE 6****FIGURE 9**

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## FIGURE 7

<A-----<A1-----  
 MC58 1 MFKRSVIAMACIFALSACGGGGGGSPDVKSADTLSPAPVSEKETEAKEDAPQAGSQG  
 2996 1 MFERSVIAMACIFALSACGGGGGGSPDVKSADTLSPAPVVAEKETEAKEDAPQAGSQG

-----<A2-----  
 MC58 61 QGAPSAQGSQDMAAVSEENTGNGGAVTADNPKNEDEVAQNDMPQNAAGTDSSTPNHTPDP  
 2996 61 QGAPSTQGSQDMAAVSAENTGNGGAATTDKPKNEDEGPQNDMPQN.....

-----<A3-----  
 MC58 121 NMLAGNMENQATDAGESSQPANQPDMANAADGMQDDPSAGGQNAAGNTAAQGANQAGNNQ  
 2996 106 .....SAESANQAGNNQ

-----A><B-----  
 MC58 181 AAGSSDPIPASNPAPANGGSNFGRVDLANGVLIDGPSQNTLTHCKGDSQNNFLDEEV  
 2996 118 PADSSDSAPASNPAPANGGSNFGRVDLANGVLIDGPSQNTLTHCKGDSQNGDNLDEEA

-----B>-----  
 MC58 241 QLKSEFEKLSADKISNYKKDGKNDKFVGLVADSVQMKGINQYTIIFYKPK..PTSFAFRFR  
 2996 178 PSKSEFENLWESERIEKYKKDGKSDKFTNLVATAVOANGTMYVLIYKDKSASSSSARFR

-----<C-----  
 MC58 299 RSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSNIFAPEGNYRYLTYGAEKLPGG  
 2996 238 RSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSNIFAPEGNYRYLTYGAEKLPGG

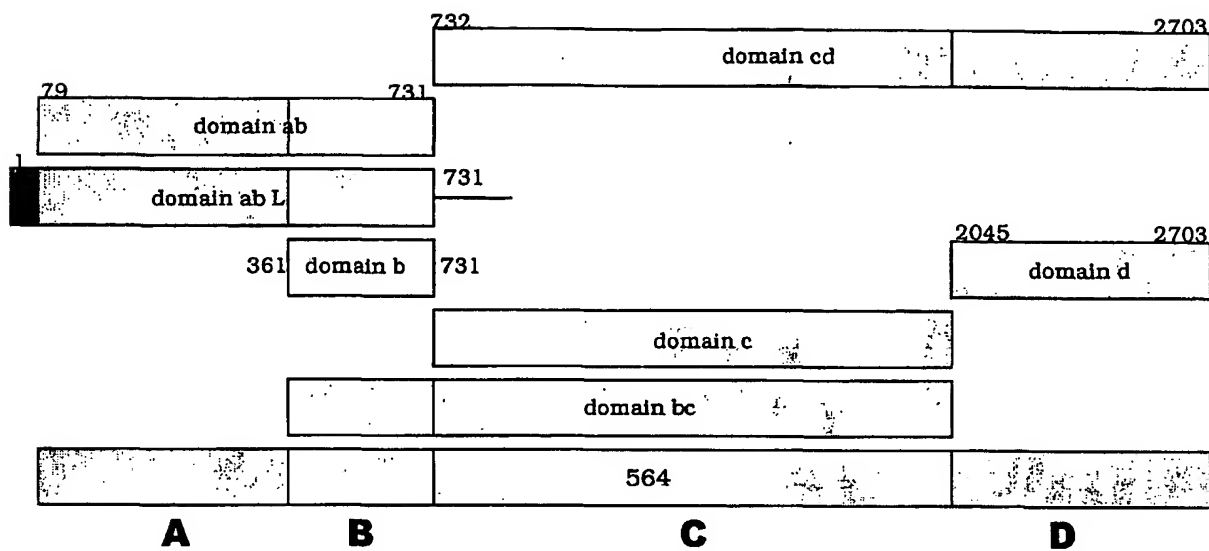
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 MC58 359 SYALRVQGEPAKGEMLAGTAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGSKSVDGIIDS  
 2996 298 SYALRVQGEPAKGEMLAGTAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGSKSVDGIIDS

-----  
 MC58 419 GDDLHMGTOKFKAIDGNGFKGTWTENGSGDVSGKFYGPAGEEVAGKYSYRPTDAEKGGF  
 2996 358 GDDLHMGTOKFKAIDGNGFKGTWTENGSGDVSGRFYGPAGEEVAGKYSYRPTDAEKGGF

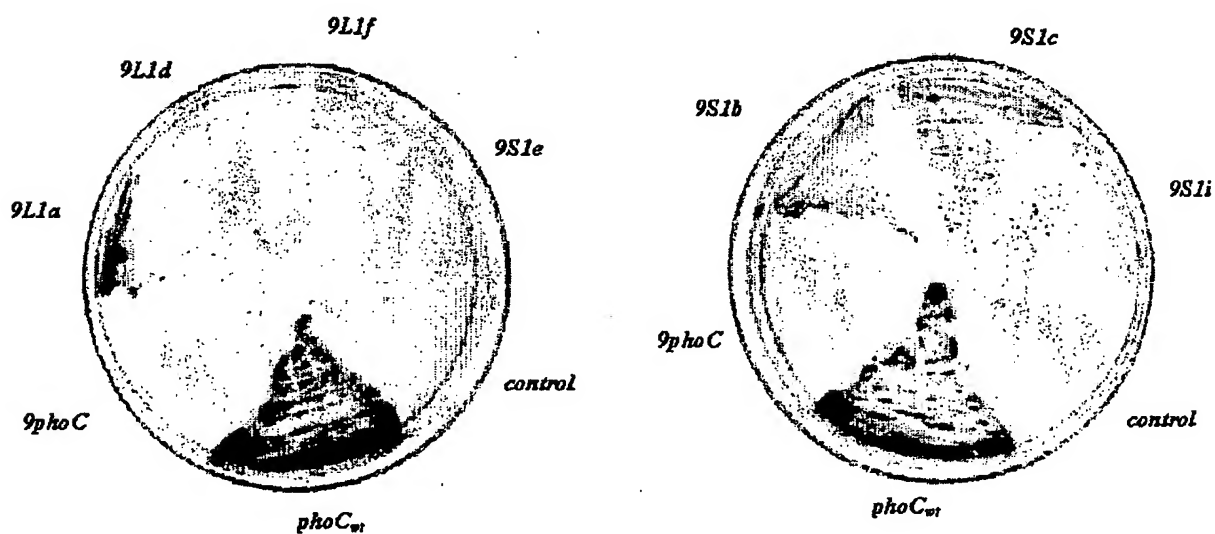
-----C>-----  
 MC58 479 GVFAKKKEQD\*  
 2996 418 GVFAKKKEQD\*

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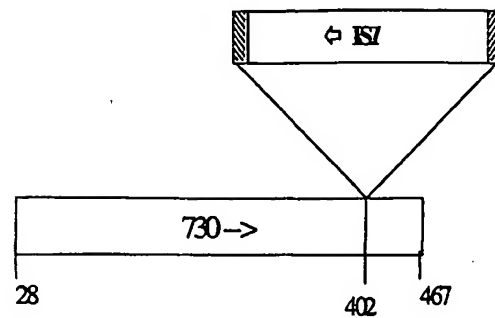
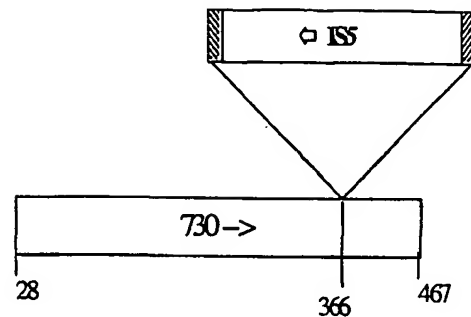
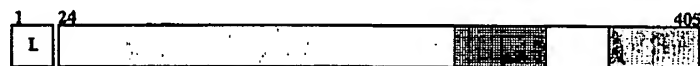
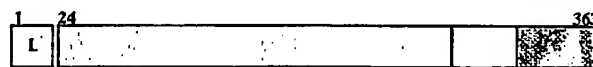
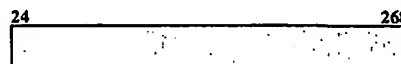
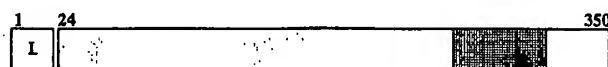
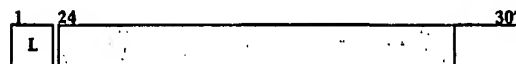
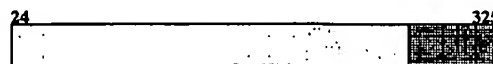
**FIGURE 8**



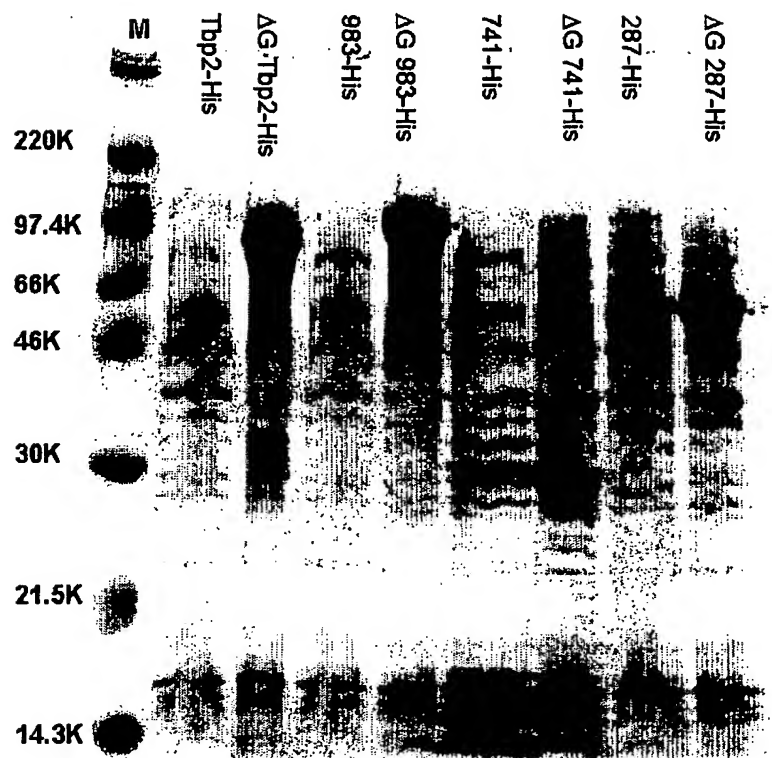
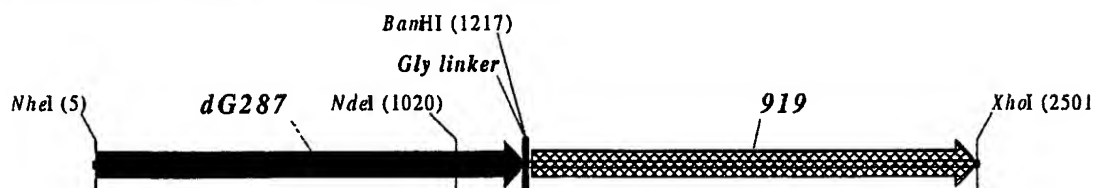
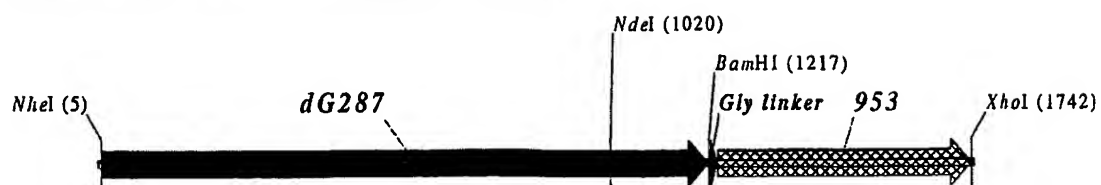
**FIGURE 10**



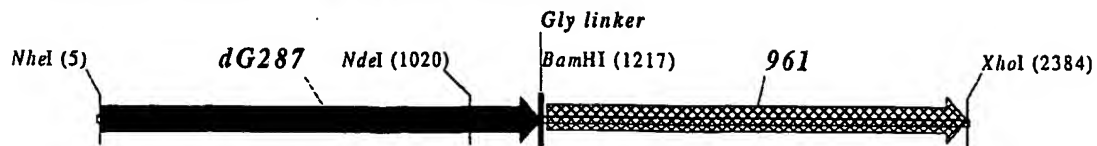
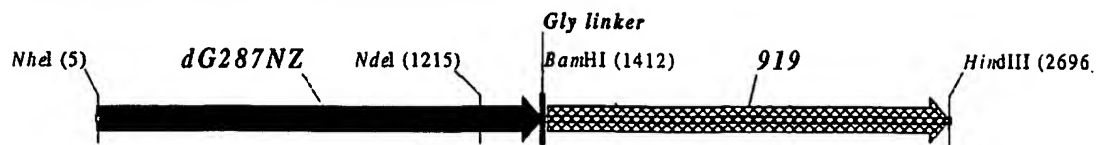
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**FIGURE 11A****FIGURE 11B****FIGURE 12****961 (2996)**961 L (2996) ☒**961 (MC58)**961 L (MC58) ☐**961a (2996=MC58)****961b (2996)****961c (2996)**961c-L (2996) ☐**961c (MC58)**961c-L (MC58) ☐**961d (2996)****961-Δ1 (2996)**961Δ1-L ☐☐ Leader Peptide☒ Region present in 2996,  
not in MC58☐ Coil-coiled segment☒ Membrane anchor

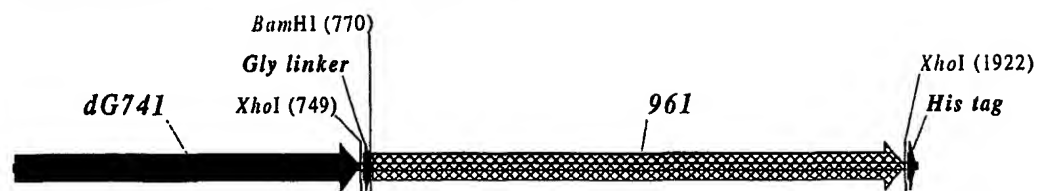
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**FIGURE 13****FIGURE 14****FIGURE 14A — ΔG287—919****FIGURE 14B — ΔG287—953**

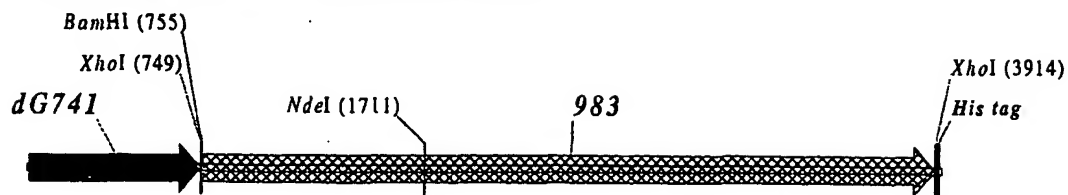
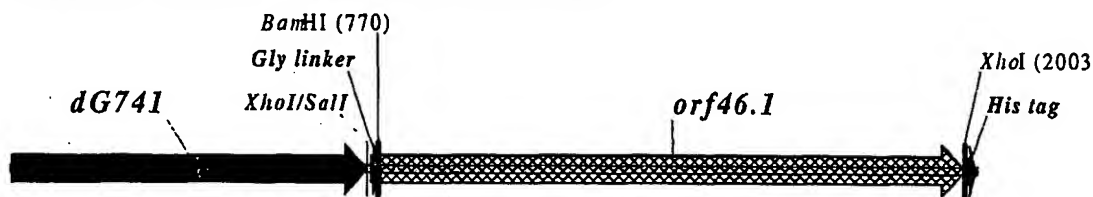
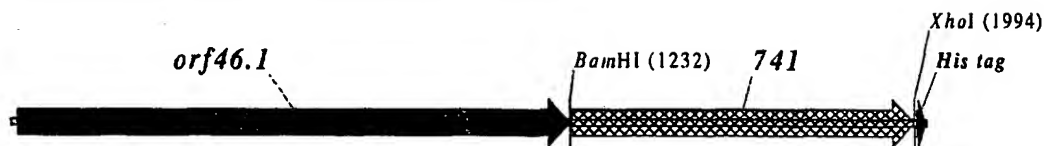
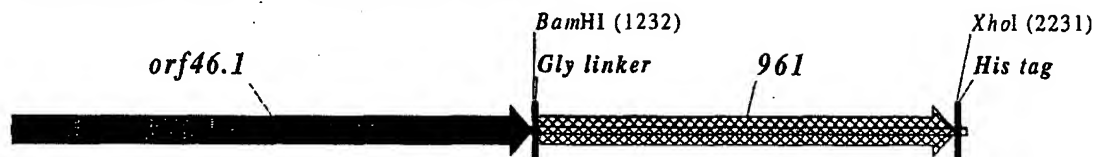
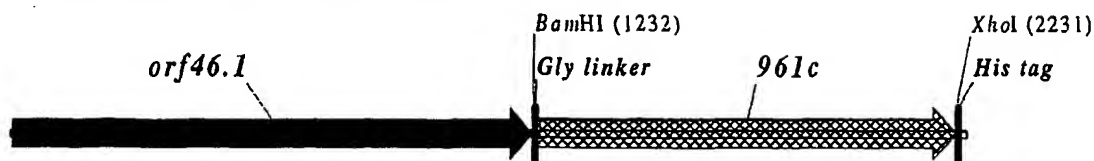
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**FIGURE 14C — ΔG287—961****FIGURE 14D — ΔG287NZ—919****FIGURE 14E — ΔG287NZ—953****FIGURE 14F — ΔG287NZ—961****FIGURE 14G — ΔG983-ORF46.1**

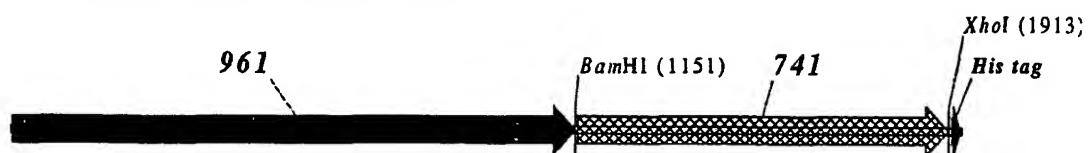
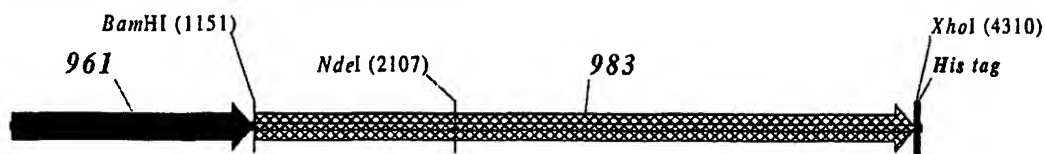
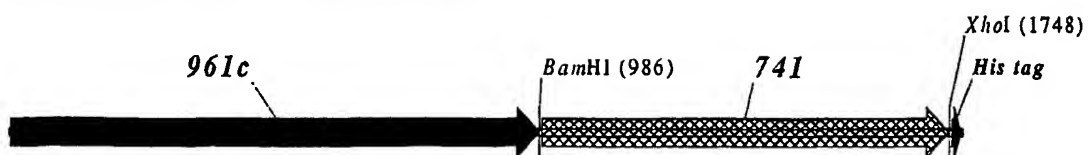
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**FIGURE 14H — ΔG983-741****FIGURE 14I — ΔG983-961****FIGURE 14J — ΔG983-961c****FIGURE 14K — ΔG741-961****FIGURE 14L — ΔG741-961c**

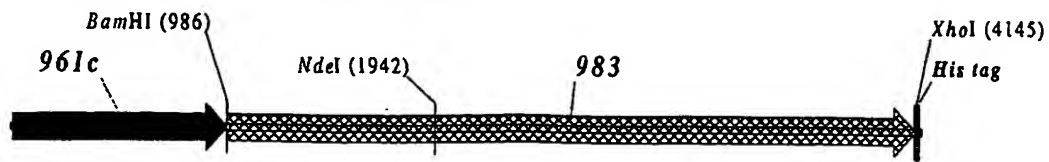
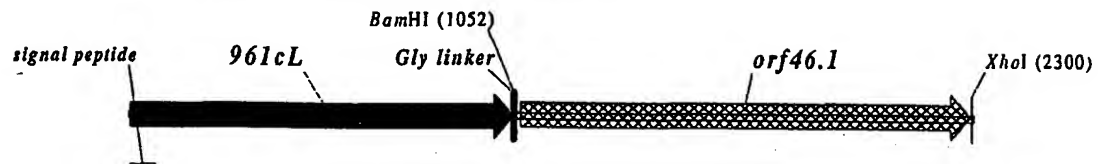
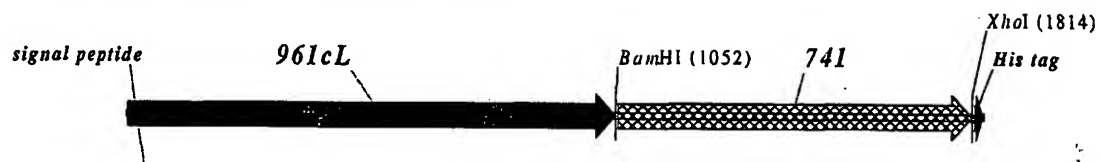
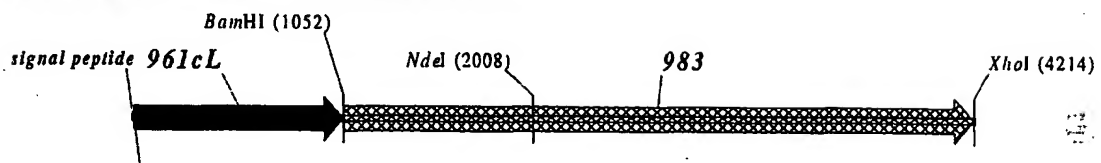
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**FIGURE 14M —  $\Delta$ G741-983****FIGURE 14N —  $\Delta$ G741-ORF46.1****FIGURE 14O — ORF46.1-741****FIGURE 14P — ORF46.1-961****FIGURE 14Q — ORF46.1-961c**

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**FIGURE 14R — 961-ORF46.1****FIGURE 14S — 961-741****FIGURE 14T — 961-983****FIGURE 14U — 961c-ORF46.1****FIGURE 14V — 961c-741**

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**FIGURE 14W — 961c-983****FIGURE 14X — 961cL-ORF46.1****FIGURE 14Y — 961cL-741****FIGURE 14Z — 961cL-983**



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